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Effects of Electrical Stimulation, Hot-Boning and Mixing vs Tumbling on the Physical and Chemical Properties of Beef Logs

M. L. JONES, E. E. RAY, J. D. THOMAS, and H. M. TSAO

ABSTRACT

Paired sides of 20 steer carcasses were used to determine the effects of electrical stimulation (ES), hot-boning (HB), and mixing vs tumbling on the physical and chemical properties of restructured beef logs. Proximate analysis data showed HB to increase water-holding capacity and ash content over cold-boning (CB). Electrical stimulation increased peak force (PF) values over nonstimulation (NS), but there was no difference in the distance to peak force (DPF) values or energy values, indicating a slight improvement in binding ability with ES. Electrical simulation and HB increased binding ability of the meat proteins. Results of this study suggest that tumbling should be used with HB and mixing should be used with CB to reduce moisture losses.

INTRODUCTION

THE MEAT INDUSTRY is seeking methods to more efficiently produce an economical, high-quality product. One way to accomplish the above is to reduce energy consumption through accelerated processing. Hot-boning increases production turnover by removing the waste bone and fat before chilling, thereby reducing inventory expenses, energy requirements, refrigeration space, transportation costs and chilling time (Henrickson, 1975). The increased toughening with hot-boning is minimized by electrical stimulation (Gilbert and Davey, 1976; Berry et al., 1980; Corte et al., 1980; Griffin et al., 1982; Bowles et al., 1983; Lyon et al., 1983).

Today's health conscious consumers are not only looking for lower prices in their meat purchases, but for a low-fat, convenience item that is easy to prepare. The meat industry is working on restructured meat products in which the fat content can be carefully monitored, and the size of the product can be controlled to meet the requirements of smaller families. Meat chunks from hot-boned (HB) and electrically stimulated carcasses may be used in these restructured products, so it would be beneficial to determine the effects of these processes on the final product. Hot-boning increases binding ability of meat proteins by increasing the amount of extractable salt-soluble proteins (SSP) and, thus, the emulsifying capacity (EC) of the raw materials (Van Eerd, 1972; Abu-Baker et al., 1982; Terrell et al., 1982a; West, 1983; Motycka and Bechtel, 1983; Choi et al, 1984; Huffman et al., 1984). Electrical stimulation decreases the amount of SSP and the EC in HB meat, but not below that of cold-boned meat (Terrell et al., 1982a; Choi et al., 1984). These restructured products are mechanically treated with such processes as mixing or tumbling to enhance the ability of meat chunks to bind together in forming a whole muscle-like roast. This product can be precooked, as the pork industry does with boneless hams, to save the consumer considerable time and energy.

This study was conducted to determine the effects of electrical stimulation, hot-boning and method of preparation on the processing properties of restructured beef log rolls.

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MATERIALS & METHODS

Beef log preparation

Twenty Longhorn and Jersey crossbred steers were slaughtered. The semitendinosus muscle (ST) from one side of each carcass (20 sides) was excised within 1 hr post-exsanguination (HB), while the remaining ST muscles (Fig. 1) were excised 48 hr post-exsanguination (CB). The right side of each animal was electrically stimulated (ES - 1 sec on, 1 sec off, 100 V, A.C., 1-2 amps) within 1 hr post-exsanguination (PE) prior to excision of the ST muscle. The pH of each side was determined after ES on all sides, and after 24 and 48 hr on CB sides. The pH was determined by blending a 10-gm sample meat from the ST in 50 mL distilled water. This solution was allowed to stand for 1 min before a reading was taken with an Orion® (model 701 A) pH meter.

Subsequently, the ST was ground through a 10 × 48 mm kidney plate on a Butcher Boy® (Model TCA 32) grinder. The meat chunks were then tumbled or mixed with a solution containing 12% distilled water, 1% NaCl and 0.4% tripolyphosphate by weight. Vacuum tumbling was done for 1 hr intermittently (10 min on - 10 min off) in a Vortron® vacuum tumbler, while mixing was done for 10 min continuously in a Betcher® (Model 150F) mixer. The weight of each sample mix averaged 1.91 ± 0.22 kg.

All mixtures were stuffed into Union Carbide® 7 cm fibrous casings, clipped at 14 kg pressure using a Tipper Tie® (Model PR4651) clipper, and hung from one end in a Koch® (Model 32 00 45) environmentally controlled (40% humidity) smokehouse. The logs were cooked with steam at 46°C for the first hr, 52°C for the second hr, 57°C for the third hr, 63°C for the fourth hr, and 91°C until an internal temperature of 66°C was reached. The temperature was monitored with a probe inserted into the center of one of the beef logs. Immediately after cooking, the beef logs were showered with cool water for 30 min, chilled at 2°C for 24 hr, wrapped in freezer wrap and frozen at − 18°C until further analyses.

Proximate analysis

Approximately 1 kg of each log was ground through a hand grinder (4 mm hole size) for moisture, ash, protein, fat and NaCl determination. Proximate analysis was determined using AOAC (1980) guidelines. NaCl content was determined using Quantab® chloride titrators (No. 1177) as directed.

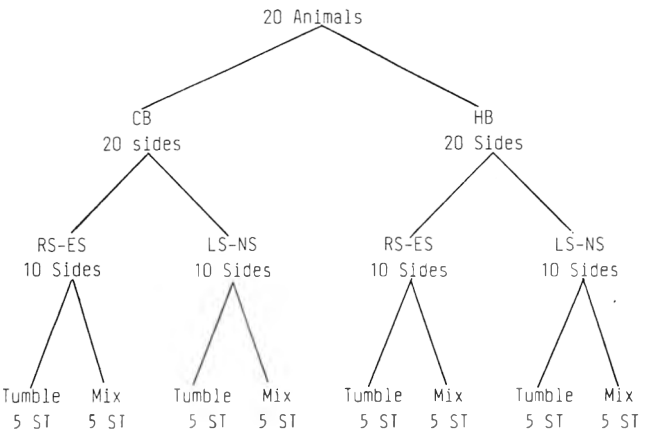

Fig. 1—Experimental design.

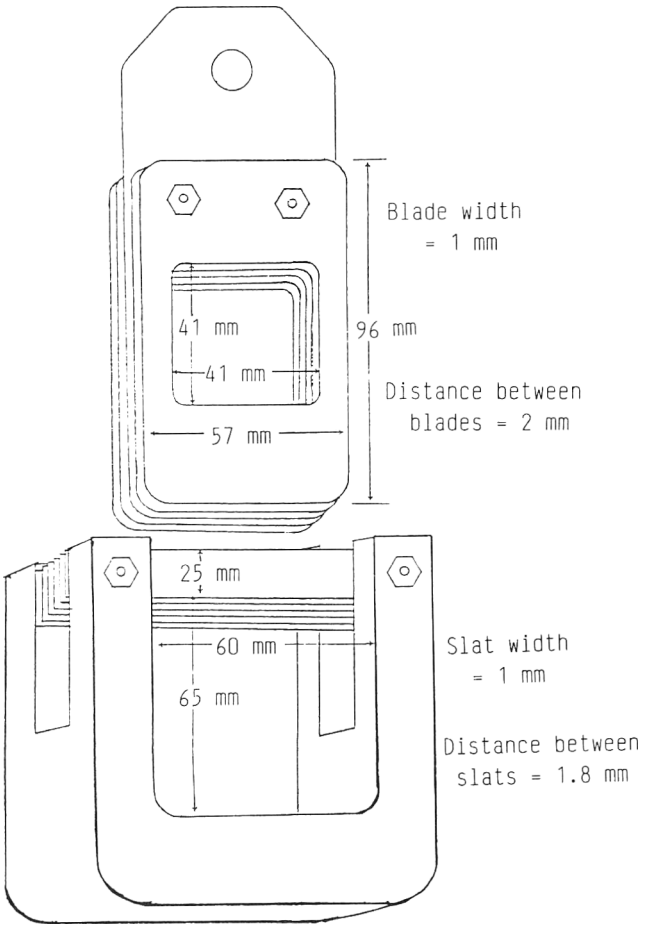

Fig. 2—Modified Kramer shear device.

Shear device

A modified Kramer shear was designed to determine tenderness values on restructured beef logs (Fig. 2). The shear device consists of five 1 mm thick blades, 57 mm wide and 96 mm long with a 41 × 41 mm square hold centered in each blade. These blades were pulled through five 1.8 mm wide slots formed from six 1 mm wide slats. The blades and the slats were made of stainless steel and were attached to the top and base of an Instron® (Model 1122, 500 kg reversible load cell) Warner-Bratzler shear device.

Instron® analysis

Five 1 cm^2 strips were cut from each log while frozen and allowed to thaw for 3 hr at 13°C. Each strip was sheared twice using the modified Kramer shear device for restructured products. The blades were pulled through the sample at a rate of 250 mm/min with a load scale setting of 50 kg and a chart speed of 200 mm/min. Force deformation curves and integrator values for the area under the curve were recorded, and the distance to the peak force (DPF) and the peak force (PF) were measured on each curve (Fig. 3). The total energy needed to shear the sample was calculated using the formula provided by Instron Co.

Statistical analysis

Data were analyzed as a completely randomize design with a 2 × 2 × 2 factorial arrangement of treatments (Fig. 1) using analysis of variance (Steel and Torrie, 1960). Tests were computed for significant main effects and any interactions among treatments. Means were separated by protected least significant differences.

RESULTS & DISCUSSION

Proximate analysis

Proximate analysis means of CB vs HB sides are presented in Table 1. The percentage of NaCl and crude fat (ether extract-

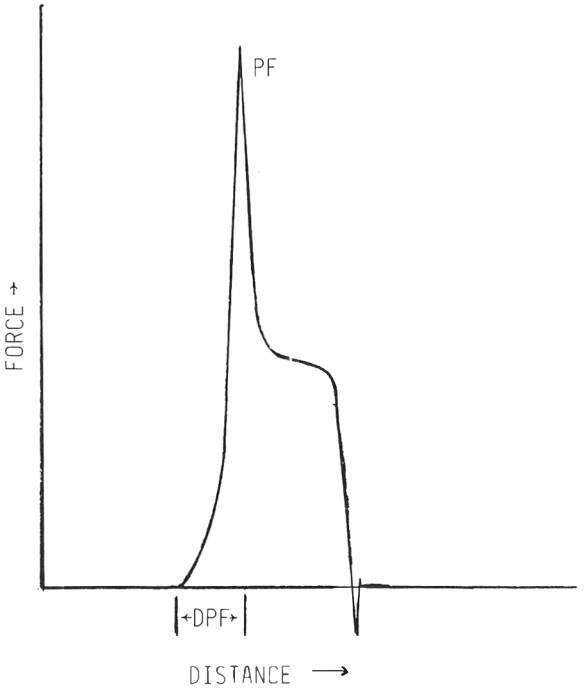

Fig. 3—Typical Instron Kramer shear force-deformation curve showing distance to peak force (DPF) and peak force (PF) values.

Table 1—Means for proximate analysis data (%) on a dry (d) and wet (w) weight basis for cold-boned (CB) vs hot-boned (HB) samples (n = 20)

Item	CB	HB	S.E.[a]
Ash-d	5.18[b]	6.33[c]	0.25
NaCl-d	1.06	0.99	0.03
NaCl-w	0.42[e]	0.38[d]	0.01
CP-d[f]	53.71	57.75	1.51
EE-d[g]	39.18	35.01	1.54
EE-w	15.59[e]	13.38[d]	0.78
Moisture	60.49[e]	62.19[d]	0.04

[a] Standard error
[b,c] Row means with different superscripts differ ($P<0.01$)
[d,e] Row means with different superscripts differ ($P<0.05$)
[f] Crude protein
[g] Ether extract or fat protein

EE) on a wet weight basis (w) were greater ($P<0.05$) for CB than for HB samples. Expressing these results on a dry weight basis (d) resulted in no significant differences, because the HB samples had a higher ($P<0.05$) amount of moisture than the CB samples. These results confirm findings of other researchers who found HB meat to have a higher water-holding capacity (WHC) than CB meat (Kastner and Russell, 1975; Berry et al., 1980; Corte et al., 1980; ray et al., 1980; 1982; Taylor et al., 1980; Griffin et al., 1981; 1982; Hamm, 1982; Motycka and Bechtel, 1983).

Percentage ash in HB meat was higher ($P<0.01$) than in CB samples, which could result from the higher moisture retention in the HB meat and, thus, more ash particles in the beef log. There were no other differences ($P>0.05$) in the proximate analysis of the product as affected by the various treatments and their interactions.

Instron® analysis

Means for the modified Kramer shear data showed (Table 2) peak force values for ES sides were less ($P<0.05$) than those for nonstimulated (NS) sides. Higher Kramer shear readings could indicate decreased tenderness or increased binding ability of the proteins in the beef logs. There is generally little problem with tenderness in a restructured product, so most of the increases in Kramer shear values have been attributed to increased binding ability of the meat proteins. These results

Table 2—Means for modified Kramer shear values for electrically stimulated (ES) vs nonstimulated (NS) samples (n = 200)

Item	ES	NS	S.E.[a]
PF (kg/cm)[b]	15.22[c]	16.36[d]	0.37
DPF (mm)[e]	11.85	11.73	0.09
Energy (Joules)	196.02	201.49	4.79

[a] Standard error
[b] Peak force
[c,d] Row means with different superscripts differ (P<0.05)
[e] Distance to peak force

Table 3—Means for modified Kramer shear values for mixing (M) vs tumbling (T) within cold-boned (CB) and hot-boned (HB) samples (n = 100)

	CB		HB		
Item	M	T	M	T	S.E.[a]
PF (kg/cm)[b]	13.55[c]	14.79[d]	18.38[e]	16.44[d]	0.53
DPF (mm)[f]	12.13[i]	11.10[g]	12.20[i]	11.73[h]	0.12
Energy (Joules)	164.66[c]	181.20[c]	239.23[e]	209.93[d]	6.77

[a] Standard error
[b] Peak force
[c,d,e] Row means with different superscripts differ (P<0.01)
[f] Distance to peak force
[g,h,i] Row means with different superscripts differ (P<0.05)

indicate that NS meat has superior binding ability than ES samples, which confirms findings of other researchers who reported that ES decreased SSP, EC, and bind in HB meat (Terrell et al., 1982a; Choi et al., 1984).

When comparing mixing vs tumbling, or CB vs HB, no (P>0.05) influence on the binding ability of the meat proteins was found. Interactions for Kramer shear values between ES vs NS sides and CB vs HB sides, ES vs NS sides and mixing (M) vs tumbling (T), or a combination of all three, were also not significant. Means for Kramer shear values of CB vs HB sides and mixing vs tumbling as an interaction are presented in Table 3. These figures show hot boning did not increase binding ability as much with tumbling as it did with mixing. Also, HB-M samples generally had higher PF values than CB samples, but there was no difference (P>0.05) between CB and HB samples when tumbled.

Distance to peak force values indicate there was no difference (P>0.05) between CB and HB samples when mixed; however, HB-M samples did have greater DPF values than HB-T samples. Mixing resulted in higher DPF values (P<0.01) in both CB and HB samples than tumbling, which suggests mixing resulted in a greater binding ability than tumbling.

Hot-boned samples had higher (P<0.01) energy values than CB samples in both tumbling and mixing, whereas there was no difference in the binding ability of CB samples. There was no (P>0.05) difference between mixing and tumbling within CB samples, but HB-M samples had higher (P<0.01) energy values than HB-T samples, indicating HB-M samples have a higher binding ability than HB-T or CB samples.

Overall, the effects of mixing vs tumbling within CB and HB sides indicate an interaction for all the Kramer shear data. Generally, hot boning yielded a superior bind than cold boning. These results agree with other researchers (Van Eerd, 1972; Abu-Baker et al., 1982; Terrell et al., 1982a; West, 1983; Motycak and Bechtel, 1983; Choi et al., 1984), who found HB meat generally had higher SSP and EC than CB meat, thus having a higher binding ability. Mixing HB meat seems to yield a superior bind than tumbling, which is probably the result of the increased surface area produced by the tearing action of the mixer. No previous research has been reported comparing mixing vs tumbling of HB raw materials.

Moisture losses

Means for moisture loss (Table 4) indicate a higher (P<0.01) cooking loss in CB than HB samples. This agrees with other researchers who have shown lower cooking losses in HB than

Table 4—Cook and drip moisture losses for cold-boned (CB) vs hot-boned (HB) samples when cooked to 66°C (n = 20)

Item	CB	HB	S.E.[a]
Cook loss, %	11.96[c]	9.73[b]	0.575
Drip loss, %	10.21	10.96	0.146
Total loss, %	20.95[e]	19.63[d]	0.521

[a] Standard error
[b,c] Row means with different superscripts differ (P<0.01)
[d,e] Row means with different superscripts differ (P<0.10)

Table 5—Cook and drip moisture losses for electrically stimulated (ES) vs non-stimulated (NS) samples when cooked to 66°C (n = 20)

Item	ES	NS	S.E.[a]
Cook loss, %	11.39	10.31	0.575
Drip loss, %	10.37[b]	10.81[c]	0.146
Total loss, %	20.58	20.01	0.521

[a] Standard error
[b,c] Row means with different subscripts differ (P<0.05)

Table 6—Cook and drip moisture losses on mixing (M) vs tumbling (T) and cold-boned (CB) and hot-boned (HB) samples (n = 100)

	CB		HB		
Item	M	T	M	T	S.E.[a]
Cook loss, %	11.30	12.63	9.45	10.02	0.813
Drip loss, %	9.96[b]	10.47[bc]	11.16[d]	10.76[cd]	0.206
Total loss, %	20.13	21.77	19.57	19.70	0.737

[a] Standard error
[b,c,d] Row means with different superscripts differ (P<0.05)

Table 7—Means for pH and pH changes on electrically stimulated (ES) vs nonstimulated (NS) sides from cold-boned carcasses (n = 20)

Item	ES	NS	S.E.[a]
pH at 0 hr	5.67[d]	5.77[e]	0.044
pH at 24 hr	5.37[b]	5.52[c]	0.036
pH at 48 hr	5.41	5.41	0.027
decline in pH after 24 hr	0.30	0.25	0.050
decline in pH after 48 hr	0.26[d]	0.37[e]	0.044

[a] Standard error
[b,c] Row means with different superscripts differ (P<0.01)
[d,e] Row means with different superscripts differ (P<0.10)

CB meat (Ray et al., 1980; 1982; Griffin et al., 1981; 1982; Motycka and Bechtel, 1983). Drip loss was higher for HB than CB samples, but there was a significant interaction with mixing and tumbling that will be discussed later. Total moisture losses for CB samples were higher (P<0.10) than HB samples, indicating the moisture saved during cooking was greater than the amount lost during storage. These findings support the proximate analysis data, which showed HB samples to have a higher moisture content (%) than CB samples.

Means for moisture loss (Table 5) indicate drip loss was less (P<0.05) for ES than NS samples. This is contrary to most findings (Jeremiah and Martin, 1980; Taylor et al., 1980; Berry and Kotula, 1982; Terrell et al., 1982b), which show ES increased or had no effect on drip loss. Cooking losses (%) and total moisture losses (%) were not affected by stimulation, which confirms other findings (Smith et al., 1979; Riley et al., 1980; Griffin et al. 1981; Terrell et al., 1982a,b). Neither mixing nor tumbling had a significant effect on moisture losses. Interactions between ES vs NS sides and mixing vs tumbling, ES vs NS sides and CB vs HB sides, or a combination of all three, were not significant.

A significant CB vs HB by mixing vs tumbling interaction (Table 6) indicates mixing increased drip losses in HB samples when compared with CB samples, while tumbling had no significant effect on drip loss between HB and CB samples. These results indicate mixing would be better to use with CB meat, and tumbling would be best for HB meat. HB samples had a higher (P<0.05) drip loss (%) when mixed than CB samples,

while drip losses for HB-T and CB-T were not ($P>0.05$) different. Therefore, hot boning generally increased drip losses for both mixing and tumbling. West (1983) indicated that moisture losses for HB meat at various points in its processing may be compensatory in nature because advantages are shown at initial points of processing (i.e. purge losses), but not later (i.e. cook losses); however, there doesn't seem to be any problem with HB meat in terms of WHC.

pH differences

Means for pH and pH changes of ES vs NS sides are presented in Table 7. The initial pH after stimulation was lower ($P<0.01$) for ES sides than for NS sides. These results agree with those of many workers (Chyrstall and Hagyard, 1976; Davey et al., 1976; Gilbert and Davey, 1976; Dutson et al., 1977; Bouton et al., 1978; 1980; Smith et al., 1979; Sonaiya et al., 1982; Terrell et al., 1982a; Choi et al., 1984), who found electrical stimulation lowers pH by increasing the rate of anaerobic glycolysis. The pH at 24 hr PE for ES sides was lower ($P<0.01$) than NS sides and had reached its lowest point, whereas the pH for the NS sides continued to decrease. The rise in pH from 24 to 48 hr in ES sides was not significant. At 48 hr PE, the pH for ES and NS sides was the same. During the first 24 hr, the decline in pH for ES vs NS sides was not significantly different, but by 48 hr the decline in pH for the NS sides was larger ($P<0.10$) than that for the ES sides. This difference (0.26 vs 0.37) was due to the lower initial pH of ES sides than the NS sides. Mixing or tumbling had no ($P>0.05$) effect on pH or pH changes.

SUMMARY

MEAT from HB sides exhibited a higher WHC than CB sides, as demonstrated by the proximate analysis and moisture loss results. Increased moisture retention resulted in higher ash retention in the HB meat. Instron analyses using PF, DPF and energy values showed HB sides to have a higher binding ability of the meat proteins, signifying an increased protein extraction in HB meat.

Electrical stimulation increased binding ability, indicating some improvement or, at least, no decrease in the possible extraction of SSP. Electrically stimulated sides had a lower moisture loss during storage (i.e. drip loss) than NS sides, but no difference in total moisture losses, indicating little or no effect on moisture loss from electrical stimulation.

Mixing (M) in combination with hot boning had higher drip losses (%) than CB sides, while M in combination with cold-boning had lower drip losses (%) than HB sides, indicating tumbling should be used with hot-boning and M should be used with cold-boning to reduce moisture losses during storage.

Electrical stimulation increased anaerobic glycolysis, and caused the initial and 24 hr pH values of ES sides to be lower than NS sides, resulting in a more rapid pH decline at 48 hr for NS sides than for ES sides.

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A Comparison of Growth of Individual Meat Bacteria on the Lean and Fatty Tissue of Beef, Pork and Lamb

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ABSTRACT

Growth of *Hafnia alvei, Serratia liquefaciens, Lactobacillus plantarum, Lactobacillus curvatus* and *Leuconostoc mesenteroides* on inoculated lean and fat tissue of beef, pork and lamb was investigated. Increases in *H. alvei, S. liquefaciens* and *L. plantarum* counts on refrigerated beef and pork fat usually were greater ($P<0.05$) than on comparable lean samples. On dark-firm-dry (DFD) beef, these differences in counts between lean and fat samples were smaller or disappeared. Increases in *L. mesenteroides* and *L. curvatus* counts on fat were either smaller than or did not differ from those on lean samples. Sour, buttermilk-like off-odors were the most common defect on stored samples.

INTRODUCTION

UNTIL RECENTLY, microbiological studies on refrigerated fresh red meat were focussed primarily on microbial activities of normal muscle tissue of beef, pork and lamb. No attempt will be made to review this topic since a summary of current information has been presented by Gill (1982). Less information is available about microbial activities on fat surfaces of refrigerated fresh red meat. Activities of microbial lipases on natural fats, including animal fats such as lard, have been described by Alford et al. (1971). Fat surfaces comprise a significant part of beef, pork and lamb carcasses and cuts. For example, most of the surface of a freshly dressed carcass consists of subcutaneous fat. Hence, this tissue is most likely one of the first to be contaminated during the slaughter-dressing operations. In the fabrication of wholesale and retail cuts, interior meat surfaces not yet exposed to microorganisms become contaminated with microbial populations residing on surface fat. Fat surface areas of retail cuts such as T-bone steaks, pork loin chops and lamb loin chops may constitute as much as 25–30% of the total surface area (Savell and Vanderzant, 1984).

Extensive microbial populations may occur on fat surfaces of beef, pork and lamb. According to Stringer et al. (1969), bacterial counts (log/in^2) of the fat surface of beef rounds immediately after slaughter and after arrival at the retail store were 4.1 and 5.6 $\log_{10}$, respectively. Bacterial counts of the fat surface of beef ribs shipped from a meat packing plant upon arrival at the load destination were 4.1–5.2 $\log_{10}$ per cm^2 (Rea et al., 1972). Information on the growth of individual microbial species on fat surfaces in scarce. Berry et al. (1973) reported considerable increases in count when fat tissues from wholesale ribs and brisket of beef carcasses were inoculated with *Pseudomonas fluorescens* or a *Flavobacterium* species. According to Gill and Newton (1980), extensive growth of a nonlipolytic strain of *P. fluorescens* occurred on a thin agar layer placed over the fat surface of chilled lamb loins. Grau (1981) reported that *Serratia liquefaciens, Yersinia enterocolitica, Enterobacter cloacae* and *Aeromonas hydrophila* grew aerobically and anaerobically on adipose tissue removed from beef muscle (*biceps femoris*) of low pH (5.4-5.6). All four strains also grew aerobically and anaerobically on muscle tissue of high pH (6.0–6.3). However, none of the four strains grew anaerobically on beef muscle of low pH and the aeromonad also failed to grow aerobically on such muscle. Later, Grau (1983) compared microbial growth of lactic acid bacteria, *Enterobacteriaceae, Brochothrix thermosphacta* and pseudomonads on the lean and fatty surfaces of vacuum-packaged beef strip loins. Considerable increases in counts occurred on fatty tissue. During storage at 5°C, lactic acid bacteria were more numerous on the lean than on the fat, whereas *B. thermosphacta* remained more numerous on the fat after the first few days. Initially, *Enterobacteriaceae* developed faster on the fat but by day 34, counts on lean and fat were about equal. In the present study, a comparison was made of the development of a series of typical meat bacteria on the lean and fatty tissue of beef (both normal and DFD beef), pork and lamb.

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MATERIALS & METHODS

Cultures

Hafnia alvei, Serratia liquefaciens, Lactobacillus plantarum, Lactobacillus curvatus and *Leuconostoc mesenteroides* were from a collection of meat isolates from previous studies (Hanna et al., 1983; Vanderzant et al., 1982). Cultures were maintained on tryptic soy agar (TSA, Difco) slants at 25°C and were transferred weekly.

Preparation of inoculum

A loopful of culture from a 24 hr TSA slant was placed into a tube containing 10 mL sterile broth. Tryptic soy broth (TSB, Difco) was used for the gram-negative and APT broth (Difco) for the gram-positive organisms. The tubes were incubated for 18 hr at 25°C. The 18 hr broth cultures, diluted with sterile broth to yield approximately 10^4 cells per mL, were used to inoculate 100 mL volumes sterile broth in 300 mL flasks at a rate of 2%. Cells were grown under both aerobic and anaerobic conditions. Anaerobic conditions were established by replacing the air in the medium and flasks with pure nitrogen. Inoculated broth cultures were incubated at 5°C for 5 days (*L. plantarum*) or 7 days (other cultures) at which time a viable cell concentration of approximately 10^8 cells per mL was reached.

Sources and preparation of meat samples

Beef, pork and lamb samples were obtained from animals that were slaughtered and dressed using conventional procedures in the Meat Science and Technology Center at Texas A&M University. After slaughter, the carcasses were chilled for at least 48 hr at 1°C to ensure full rigor development and complete temperature decline and to achieve ultimate pH. Lean and subcutaneous fat samples from beef, pork and lamb were obtained from the top round, ham (uncured) and foresaddle, respectively. The lean and fat sections that were removed from the carcasses were wetted thoroughly with 70% alcohol and flamed with a gas flame. After removal of the surface tissue with sterile scalpels, samples (10 cm^2, 2 mm thick) were cut from the lean and fat tissue with sterile instruments under aseptic conditions. They were held under refrigeration in sterile petri dishes until they were inoculated with individual test organisms.

Experimental design

A total of 104 samples (52 lean and 52 fat) of each beef, pork and lamb were used to study the growth pattern of each test culture. For

the experiments comparing bacterial growth on normal versus DFD beef, 104 samples were used per test culture for each type of beef. Of each group of 52 samples, four were used to check the sterility of the experimental samples. Twenty-four of the remaining 48 samples were inoculated with aerobically grown cells and the other 24 samples were inoculated with anaerobically grown cells. Of each set of 24 samples, four samples were used to determine bacterial counts and pH at day 0. The remaining 20 samples were divided into five sets of four samples each. Two of the sets were packaged and stored in polyvinyl chloride (PVC) film, the remaining three sets were vacuum-packaged in high-oxygen barrier (HOB) film. One set of the PVC-wrapped samples was examined after 3 and 6 days of storage, and one set of the vacuum-packaged samples was examined after 7, 14, and 21 days of storage. Three of the experimental samples in each set were used for microbiological analysis, the fourth was used for measurement of pH.

Inoculation, packaging and storage of meat samples

Samples were inoculated by immersing them for a few seconds in broth containing approximately 10^3–10^4 viable cells per mL. After the excess fluid had drained off, the samples were blotted lightly with sterile paper towels. Samples were placed in sets of four in a plastic foam tray (13 × 13 × 1 cm) for overwrapping with PVC film and in HOB film bags for vacuum packaging. The PVC-wrapped samples (inoculated and uninoculated control samples) were heat sealed (Model 104-A, Heat Sealing Equipment Co., Cleveland, OH).

The PVC film (Prime Wrap II, The Goodyear Tire and Rubber Company, Akron, OH) had an oxygen transmission rate (OTR) of 6.5 l/m^2/24 hr and moisture vapor transmission rate (MVTR) of 341–419 g/m^2/24 hr. The HOB film was a laminate of 2 mil Saran coated polyethylene film and 1 mil nylon. It had an OTR of 7.8 cc/m^2/24 hr at 0% RH and 22.8°C and a MVTR of 9.3 g/m^2/24 hr at 90% RH and 37.8°C.

For vacuum packaging, the samples were packaged in HOB film using a chamber-type heat sealing vacuum packaging system (Boss, 6380 Bad Homburg 6, West Germany). The PVC-wrapped and vacuum-packaged samples were stored in the crisper drawers of a refrigerator maintained at 5°C and 98–100% relative humidity. The relative humidity was monitored using a relative humidity meter (Air-Guide, Air-Guide Instrument Co., Chicago, IL).

Microbiological examination

Each of the lean and fat samples was placed separately in a sterile Stomacher bag (Tekmar) containing 100 mL sterile 0.1% peptone broth. The samples were blended with a Stomacher-400 (Tekmar) for 1 min. One-tenth milliliter volumes of appropriate decimal dilutions (0.1% sterile peptone) were plated on prepoured sterile TSA plates using the spread-plate method. Plates were incubated at 25°C for 2 days (gram-negative cultures) or 3 days (gram-positive cultures). Counts were calculated from countable plates and were expressed per cm^2.

Recognition of defective samples

Immediately after opening the PVC-wrapped and vacuum-packaged samples at each sampling interval, they were examined by two judges for off-odors by smell and for discoloration by sight. Each of these judges had a minimum of one year on-the-job training in the recognition of off-odors and discoloration of packaged red meats. Perceptible defects when found were noted but samples were not rated numerically.

pH measurement

At each sampling interval, the pH of a lean and fat sample was measured immediately after opening the packages using a pH meter (Digital pH millivolt meter 611, Orion Research, Inc., Londonderry, NH) equipped with a surface probe (Electrode pH model 91-63, Orion Research, Inc., Londonderry, NH).

Statistical analyses

Differences in bacteriological counts of lean and fat samples within a species (beef, pork, lamb), type of inoculum (aerobically and anaerobically grown cells of a culture) and packaging procedure (PVC, HOB-film) were examined for significance by analysis of variance (SAS, 1982). When significant ($P<0.05$) main effects were observed, mean separation was accomplished by the use of Duncan's Multiple range test (Duncan, 1955).

RESULTS

INCREASES in *H. alvei* count on fat samples of beef and pork were greater ($P<0.05$) than those on comparable lean samples (Fig. 1). In contrast, increases in *H. alvei* count on fat samples of lamb were greater ($P<0.05$) than those of comparable lean samples in only two of four comparisons. Increases in *S. liquefaciens* count on fat samples of beef and pork were nearly always greater ($P<0.05$) than those on comparable lean samples (Fig. 2). Increases in *S. liquefaciens* count on fat samples of lamb usually did not differ ($P>0.05$) from those on comparable lean samples. For beef, pork and lamb samples inoculated with *L. mesenteroides* (Fig. 3), increases in count were

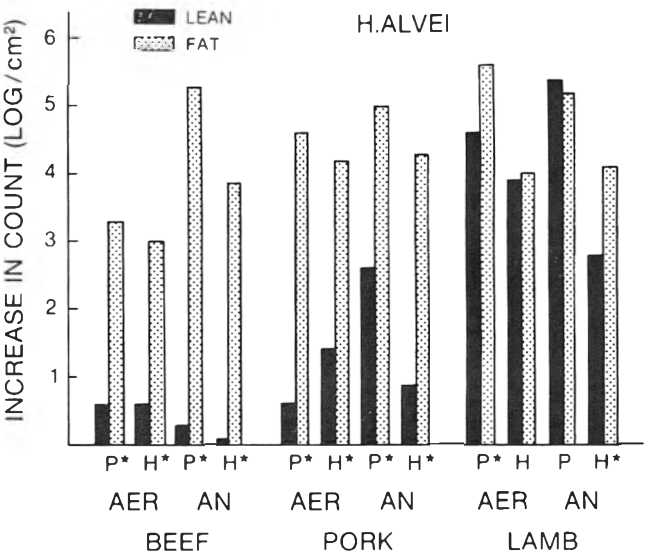

Fig. 1—Increases in count of H. alvei on lean and fat samples of beef, pork and lamb (Means of 3 samples). Samples in PVC film (P) were stored for 6 days, those in HOB film (H) were stored for 21 days at 5°C. Samples were inoculated with aerobically (AER) and anaerobically (AN)-grown cells. *Differences in increases in count between lean and fat samples within species, type of inoculum and packaging procedure were significant at $P<0.05$.

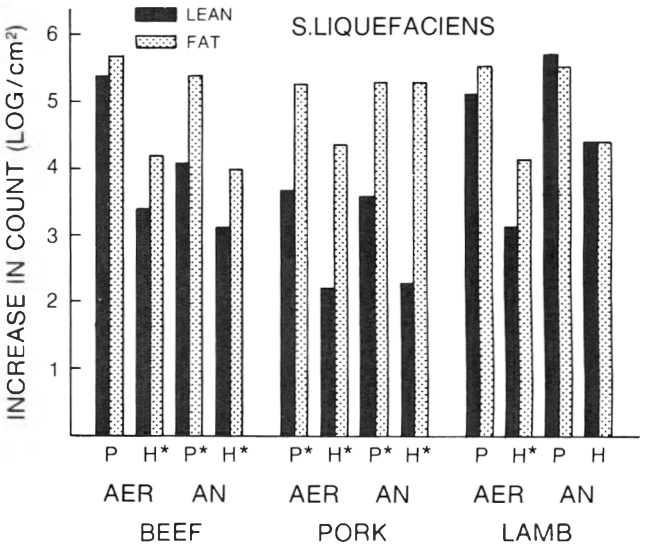

Fig. 2—Increases in count of S. liquefaciens on lean and fat samples of beef, pork and lamb (Means of 3 samples). Samples in PVC film (P) were stored for 6 days, those in HOB film (H) were stored for 21 days at 5°C. Samples were inoculated with aerobically (AER) and anaerobically (AN)-grown cells. *See Fig. 1.

greater ($P<0.05$) on lean than on comparable fat samples in 6 of 12 comparisons. In 11 of 12 comparisons, increases in *L. mesenteroides* count on lean samples were numerically greater than those on comparable fat samples. In contrast, increases in *L. plantarum* count on fat samples of beef were consistently greater ($P<0.05$) than those on comparable lean samples (Fig. 4). Increases in *L. plantarum* count on fat samples of pork packaged and stored in PVC film also were greater ($P<0.05$) than those on comparable lean samples. However, increases in *L. plantarum* count of lean and fat samples that were vacuum packaged and stored in HOB film did not differ ($P>0.05$). For lamb, increases in count on lean samples inoculated with aerobically grown cells and packaged and stored in HOB film were greater ($P<0.05$) than those on comparable fat samples; they were smaller than those on comparable fat samples when inoculated with anaerobically grown cells. Increases in count of samples packaged and stored in PVC film did not differ ($P>0.05$). Increases in *L. curvatus* count on the lean and fat samples of beef (data not shown in figure) were similar to those shown for *L. mesenteroides* (Fig. 3).

The large differences in increases in count of *H. alvei* and *S. liquefaciens* between fat and lean samples of normal beef had narrowed (*H. alvei*) or disappeared (*S. liquefaciens*) with the DFD samples (Fig. 5). For normal beef, increases in count of *L. mesenteroides* on lean samples were greater ($P<0.05$) than those on fat samples in 2 or 4 comparisons (HOB samples), for DFD beef in 4 of 4 comparisons (Fig. 6). For *L. plantarum*, increases in count on normal beef wrapped and stored in PVC film were greater ($P<0.05$) on the fat than on the lean samples, whereas for vacuum-packaged samples stored in HOB film increases in count were greater ($P<0.05$) on the lean than on the fat samples (Fig. 6). In contrast, for DFD beef wrapped and stored in PVC film, increases in count of *L. plantarum* were greater ($P<0.05$) on the lean than on the fat samples. Increases in *L. plantarum* count for lean and fat samples that were vacuum-packaged and stored in HOB film did not differ ($P>0.05$).

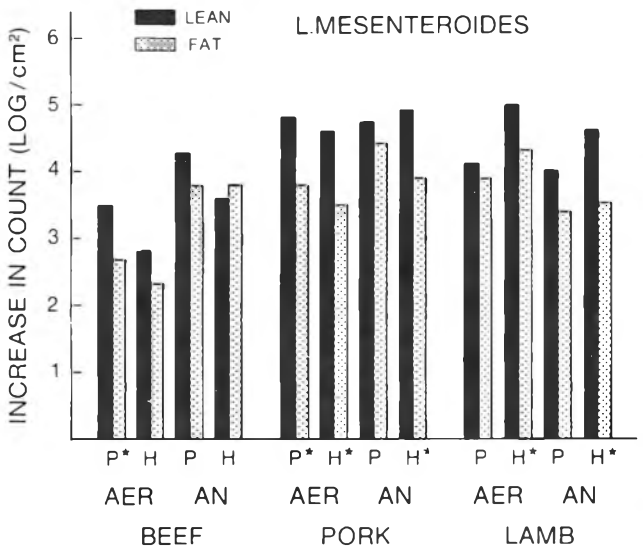

Fig. 3—Increases in count of L. mesenteroides on lean and fat samples of beef, pork and lamb (Means of 3 samples). Samples in PVC film (P) were stored for 6 days, those in HOB film (H) were stored for 21 days at 5°C. Samples were inoculated with aerobically (AER) and anaerobically (AN)-grown cells. *See Fig. 1.

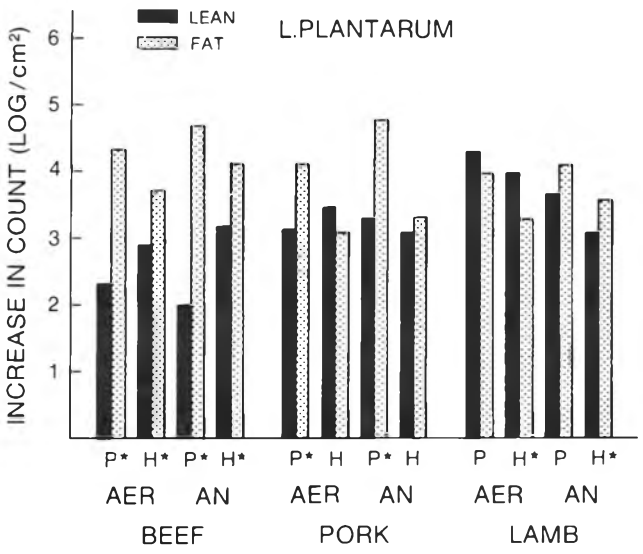

Fig. 4—Increases in count of L. plantarum on lean and fat samples of beef, pork and lamb (Means of 3 samples). Samples in PVC film (P) were stored for 6 days, those in HOB film (H) were stored for 21 days at 5°C. Samples were inoculated with aerobically (AER) and anaerobically (AN)-grown cells. *See Fig. 1.

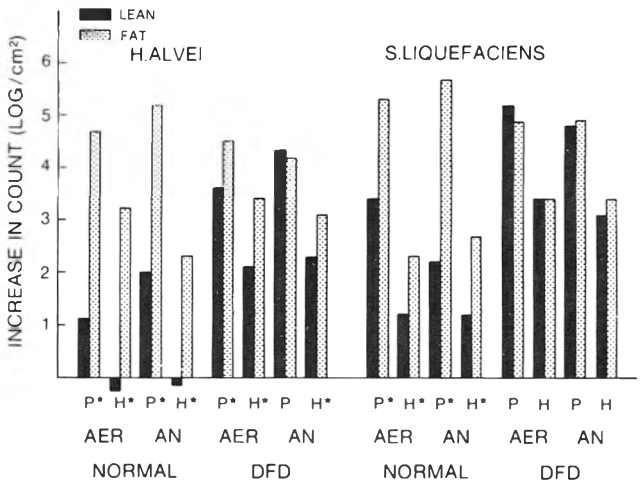

Fig. 5—Increases in count of H. alvei and *S. liquefaciens* on lean and fat samples of normal and DFD beef (Means of 3 samples). Samples in PVC film (P) were stored for 6 days, those in HOB film (H) were stored for 21 days at 5°C. Samples were inoculated with aerobically (AER) and anaerobically (AN)-grown cells. *See Fig. 1.

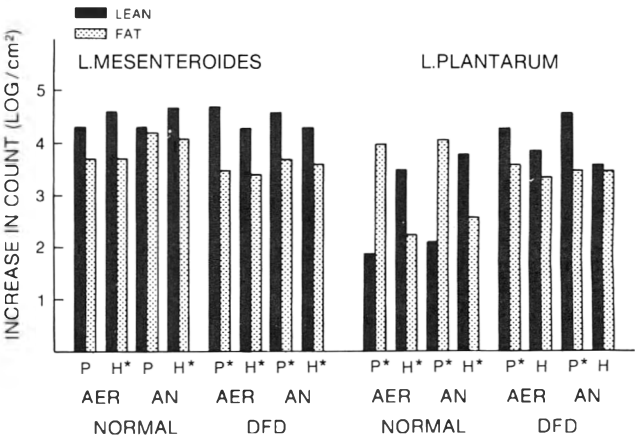

Fig. 6—Increases in count of L. mesenteroides and *L. plantarum* on lean and fat samples of normal and DFD beef (Means of 3 samples). Samples in PVC film (P) were stored for 6 days, those in HOB film (H) were stored for 21 days at 5°C. Samples were inoculated with aerobically (AER) and anaerobically (AN)-grown cells. *See Fig. 1.

In most comparisons, increases in count of test cultures on either normal or DFD samples inoculated with aerobically grown cells did not differ ($P>0.05$) from those on comparable samples inoculated with anaerobically grown cells. A comparison of increases in count of test cultures on lean samples of normal versus DFD beef showed that increases in count of *H. alvei*, *S. liquefaciens* and to some extent those of *L. plantarum* were greater ($P<0.05$) for DFD samples than for normal samples. Increases in count of *L. mesenteroides* on lean samples of normal and DFD beef usually did not differ ($P>0.05$). Patterns of increases in count of test cultures on fat samples of normal versus DFD beef were not consistent. Relationships of increases in counts of cultures on lean and fat samples at 3 days of storage in PVC film and at 7 and 14 days in HOB film showed similar trends to those described for comparable samples examined after 6 days (PVC film) or 21 days (HOB film), (see Fig. 7 for data on beef samples).

Initial pH values of lean samples were considerably lower than those of comparable fat samples. pH ranges of normal lean and fat samples were as follows: for beef, 5.43–5.61 versus 6.18–7.02; for pork, 5.32–5.98 versus 6.17–7.48; and for lamb, 5.38–6.34 versus 6.37–7.12. During refrigerated storage of inoculated (*H. alvei*, *S. liquefaciens*, *L. mesenteroides*, *L. plantarum*) normal beef, pork and lamb samples, pH changes of 0.5 or greater were observed in 29 of 96 (4 test cultures × 3 species × 2 films × 2 types of cells × 2 tissues) samples, in 27 of 29 samples these changes constituted decreases in pH. Twenty-three of these 27 samples were samples of fat. Decreases in pH of 0.5 or greater occurred in 12 of 32 DFD samples; 9 of the 12 samples were fat samples.

Buttermilk-like and sour off-odors were the only off-odors observed in nearly all (lean and fat) beef, pork, and lamb samples inoculated with *L. plantarum* and stored at 5°C for 6 days in PVC film or for 21 days in HOB film. Buttermilk-like and sour off-odors were also present in some beef, pork and lamb samples inoculated with *L. mesenteroides*. Many of the fat samples of beef, pork and lamb inoculated with *H. alvei* exhibited a distinct fishy off-odor. Samples inoculated with *S. liquefaciens* exhibited a variety of off-odors such as sour, buttermilk-like, rotten, rancid, spicy, "old pork" and "old meaty."

Green-brown discolorations were observed on (a) a few of the lean samples of beef and lamb inoculated with *H. alvei* or *S. liquefaciens* and (b) lean DFD samples inoculated with *H. alvei*, *S. liquelfaciens*, or *L. plantarum* that were packaged and stored in PVC film. The predominant off-odor observed on DFD samples (lean and fat) inoculated with *L. mesenteroides* or *L. plantarum* was a sour, buttermilk-like odor. Rancid or spicy-mustardy off-odors were observed on all DFD samples inoculated with *S. liquefaciens*. Sour, rancid or fishy off-odors were present on DFD samples inoculated with *H. alvei*.

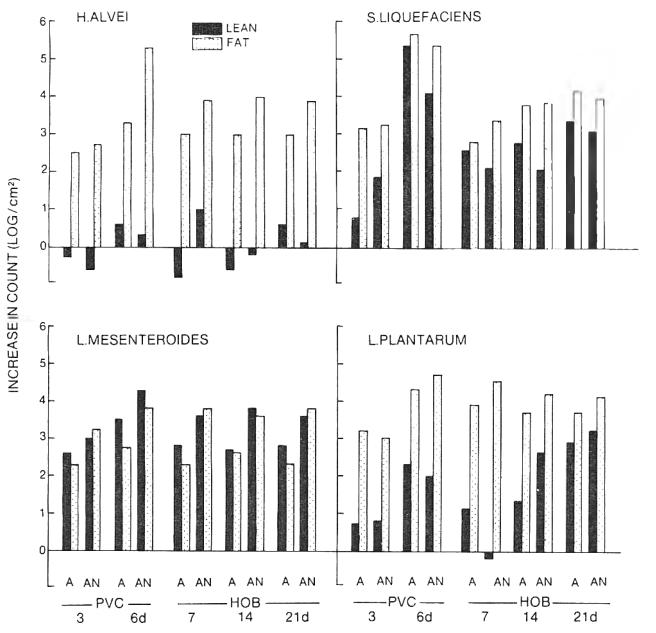

Fig. 7—Increases in count of test cultures on lean and fat samples of beef (Means of 3 samples). Samples in PVC film were stored for 3 and 6 days, those in HOB film were stored for 7, 14, and 21 days at 5°C. Samples were inoculated with aerobically (A) and anaerobically (AN)-grown cells.

DISCUSSION

THE RESULTS of the present study confirm those of Berry et al., (1973), Gill and Newton (1980) and Grau (1981, 1983), indicating that some bacteria can grow extensively on fat surfaces. According to Gill and Newton (1980), the low-molecular weight substrates available at the fat surface for bacterial growth are probably derived largely from the serum in cut blood vessels. In view of the results reported by Grau (1980, 1981), the more extensive growth of *H. alvei* and *S. liquefaciens* on adipose tissue as compared with that on muscle tissue of beef and pork probably is caused by differences in pH and lactate content of lean and fat.

Considerable increases in *S. liquefaciens* count occurred on inoculated vacuum-packaged muscle tissue of beef, pork and lamb. In contrast, no growth of *S. liquefaciens* was reported by Grau (1981). This difference in results was caused most likely because of a difference in OTR of the films. In a subsequent study, Grau (1983) reported that *Enterobacteriaceae* were able to grow on lean of low pH in the presence of some oxygen and presumably then were able to adapt to the low pH and high lactate content of the lean before conditions in the vacuum package became essentially anaerobic. This may explain why in the present study increases in count of test cultures on samples of normal lean meat inoculated with aerobically grown cells and stored in HOB film in most cases did not differ from comparable samples inoculated with anaerobically grown cells. These findings also suggest that rapid and complete evacuation of air and the use of films with very low OTR values could reduce the growth of some bacteria in vacuum-packaged beef.

In view of the results reported by Grau (1981), differences in the growth response of *H. alvei* and *S. liquefaciens* on DFD, compared to that of normal beef, most likely were caused by a narrowing of the pH difference between lean and fat tissue of the DFD samples as compared to that of the normal samples.

The predominance of sour, buttermilk-like odors of samples inoculated with *L. mesenteroides*, *L. plantarum* and *L. curvatus* agrees with commercial experience with refrigerated vacuum-packaged beef and with other reports in the literature (Hanna et al., 1979, 1983; Patterson and Gibbs, 1977; Sutherland et al., 1975). Patterson and Gibbs (1977), also reported a variety of off-odors ("burnt match, amines", "malty", "damp leather") on beef inoculated with *S. liquefaciens*. On vacuum-packaged meat of high pH, *H. alvei* produced an off-odor of "slight pickles", whereas on meat stored aerobically the off-odor was described as "cabbagey" (Patterson and Gibbs, 1977). In the present study, fishy odors predominated. Green discolorations observed on some lean samples inoculated with *H. alvei* and *S. liquefaciens* most likely were caused by H_2S production resulting in the formation of sulfmyoglobin which has a green color (Gill and Newton, 1979). *H. alvei* (Hanna et al., 1979) and *S. liquefaciens* (Patterson and Gibbs, 1977) have been associated with H_2S production.

The results reported by Grau (1983) and in the present study have certain practical applications. It is plausible that during the cooling of hot-boned beef, growth of certain bacteria such as *H. alvei* is more likely to occur on fatty tissue and on those muscles with a high pH. Because of differences in muscle pH, the amount of bacterial growth on lean tissue is likely to vary. Electrical stimulation of carcasses before hot-boning accelerates the post-mortem fall in muscle pH (Bouton et al., 1980).

—Continued on page 11

Characteristics of Dry-Cured Hams as Affected by Needle Tenderization and Vacuum Packaging

J. D. KEMP, J. D. FOX, and B. E. LANGLOIS

ABSTRACT

Needle tenderization of skinned hams accelerated salt absorption and weight loss and shortened curing time for dry-cured hams. Vacuum packaging of hams after they achieved an 18% weight loss prevented most additional weight loss thus yielding a higher percentage of moisture and a lower percentage of salt than in nonpackaged hams. Total bacterial and lactobacilli counts were higher in packaged hams but did not affect internal appearance scores or sensory evaluation scores. In general, the use of needle tenderization and vacuum packaging was helpful in reducing curing and aging time and in controlling yields in dry-cured hams.

INTRODUCTION

METHODS for accelerating the curing and aging time for dry-cured hams have included curing skinless hams (Montgomery et al., 1976), curing boneless hams (Kemp et al., 1979, 1983; Leak, 1984), tumbling hams during curing (Leak et al., 1984; Ockerman and Organisciak, 1978); and use of a mechanical tenderizer prior to curing (Kemp and Fox, 1985; Leak, 1984; Marriott et al., 1984). Hams have been produced that meet the USDA requirements for 4% salt and 18% weight loss (Mussman, 1977) in as short a time as 5wk (Kemp and Fox, 1985). Vacuum packaging of hams after they achieve the required 18% weight loss has been shown to be a plausible way to control further weight loss during aging (Kemp et al., 1981). The USDA regulations (Houston, 1985), however, require that the hams, unless approved as trichinae free by either individual examination or pooled sample technique, remain in cure for a much longer time than required to attain the salt and weight loss goals. This work was planned to develop hams that meet all requirements except time in cure for dry cured hams so the accelerated procedures can be used with trichinae free fresh hams or be the basis for developing techniques that may be acceptable for future regulations for dry cured hams.

MATERIALS & METHODS

FORTY-FOUR frozen hams were thawed 3 days at 2–4°C, skinned and trimmed of excessive fat and freezer burn spots. The ham mean weight after skinning and trimming was 7.6 kg. Half the hams were tenderized by passing them twice (once outside up and once inside up) through a Ross Blade Tenderizer. The other half were not tenderized. All hams were dry cured using 7 kg of cure per 100 kg of hams by applying one-half the cure on day 0 and one-half on day 3. The curing mixture contained 83.4% salt, 13.9% white sugar, 1.7% sodium nitrate and 0.9% sodium nitrite. Hams remained at 2–4°C for 2 wk. At this time core samples of 2.5 cm diameter and approximately 5 cm long were obtained from the cushion and shank portion of five hams of each group. The cores were divided into outside, center and inner portions and analyzed for salt and nitrite (AOAC, 1980). This process was repeated weekly for 2 wk. The core holes were filled with acetylated monoglyceride (MYVACET 7-00, Eastman Chemical). All hams (both cored and intact) were held after the 2 wk curing period in a salt equalization room at 13°C for 10 days. The intact hams were smoked one day and then held in an aging room at 24°C until the week in which the total weight loss, based on in-cure weight, equalled or exceeded 18%. At this point alternate hams (8 or 9) in each group were placed in Cryovac(®) bags from which the air was then excluded. The bags were sealed and heat-shrunk. The other 8 or 9 in each group were not bagged. They were all held 4 additional weeks after which they were examined subjectively and microbiologically for total aerobic, lactobacilli and yeast and mold counts by the swab rinse method described by Marth (1978). Hams were cut and examined subjectively for color (4 = dark red, 3 = red, 2 = light red, 1 = pink), aroma (4 = typically aged, 3 = moderately aged, 2 = slightly aged, and 1 = no apparent age), and general appearance (4 = excellent, 3 = good, 2 = fair, 1 = poor). One 2.5 cm and three 1.3 cm center thick slices were obtained. One 1.3 cm slice was trimmed of fat and analyzed for moisture, salt and nitrite (AOAC, 1980). The 2.5 cm slices were broiled and allowed to equilibrate to room temperature. A 2.54 cm core was obtained from near the approximate center of each semimembranosus (SM), semitendinosus (ST) and biceps femoris (BF) muscle and sheared with a Warner-Bratzler attachment on an Instron Universal Testing Instrument. Two 1.3 cm slices were broiled and evaluated by an experienced 10-member sensory panel for tenderness, flavor preference, flavor intensity, saltiness and overall satisfaction. Tenderness, flavor intensity and saltiness scores were on a 1 to 8 rating scale while flavor preference and overall satisfaction scores were on a 1 to 8 point hedonic scale. Data were analyzed using the GLM procedures of SAS (1984).

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RESULTS & DISCUSSION

ALL HAMS appeared normal and lost weight in line with expected standards. The mean values (Table 1) at each time period (with one exception—inner sample of cushion at week 4) for each section of the cores of the tenderized hams showed a trend toward increased salt compared to the nontenderized hams with several differences being significant. These data showed that tenderized hams absorbed salt faster than nontenderized hams in agreement with Kemp and Fox (1985). The inner portion of the cores at 4 wk contained approximately 3% salt which indicated that the hams were ready to smoke or place in an aging room. The moisture in the center of the hams at that time was approximately 65–70%. During the subsequent aging period there was additional salt absorption as well as additional moisture loss so that the final salt content of the center area approached 4% or the salt content of the whole slices exceeded 4% as required by USDA regulations (Mussman, 1977). Salt content of the top portions of the cores decreased with time while the center and inner portions increased so that the longer the time held, the more uniform the salt became throughout the hams.

Nitrite values (Table 2) followed a pattern somewhat like that of salt with a trend toward greater values for tenderized than for nontenderized hams. Since nitrite is being produced from nitrate and itself decomposes, levels can be quite variable. As time after curing increased, the values for nitrite decreased. In all periods the values were within USDA requirements but sufficiently high for proper nitrite functions.

The 34 hams that were not cored were held in aging at 24°C until the week in which their weight loss was 18% or more. At this time, half of the hams were placed in Cryovac® barrier bags which were then air evacuated and heat shrunk. Hams were then placed back in the aging room. The other half were further aged without packaging.

Table 3 shows that it took slightly longer for the nonten-

Table 1—Salt percentage in ham cores by treatment and time

	Cushion section core						Shank section core					
	Top		Center		Inner		Top		Center		Inner	
Time	Tend	Non-tend	Tend	Non-tend	Tend	Non-tend	Tend	Non-tend	Tend	Non-tend	Tend	Non-tend
2 wk	5.89	4.56	3.29	3.24	2.33	1.89	5.62[a]	2.57[b]	4.28[a]	2.17[b]	2.95[c]	1.42[d]
3 wk	6.46	4.23	4.34[e]	2.99[f]	3.14[c]	2.19[d]	4.88	3.60	3.82[e]	2.79[f]	2.93[e]	1.95[f]
4 wk	4.41	3.90	4.39	3.81	2.92	3.26	5.12	3.31	4.77[c]	2.89[d]	3.54	2.70

[ab] $p<0.01$ between treatment within time and core section.
[cd] $p<0.05$ between treatment within time and core section.
[ef] $p<0.10$ between treatment within time anc core section.

Table 2—Nitrite content (PPM) of ham cores by treatment time

	Cushion section core						Shank section core					
	Top		Center		Inner		Top		Center		Inner	
Time	Tend	Non-tend	Tend	Non-tend	Tend	Non-tend	Tend	Non-tend	Tend	Non-tend	Tend	Non-tend
2 weeks	139.2	91.3	49.9	44.5	34.2	25.9	158.3[e]	70.4[f]	68.7	50.0	44.5[e]	28.8[f]
3 weeks	92.4	61.2	30.5	38.6	26.1	21.3	64.9	51.1	63.7	33.9	35.8	42.8
4 weeks	36.3	31.7	14.5	15.3	11.0	13.1	45.9[e]	33.4[f]	27.2[c]	12.8[d]	13.4	9.6

[cd] ($p<0.05$) between treatments within time and core sections.
[ef] ($p<0.10$) between treatments within time and core sections.

Table 3—Effect of tenderizing on time in curing and aging and on yields before vacuum packaging

Item	Non-tenderized	Tenderized
Wk to reach 18% wt loss	5.0	4.7
Ham yields, %		
Cured	94.5	94.3
Salt equalization	86.3	85.6
Smoked	85.3	84.3
Ready for packaging	81.3	81.0

derized hams to achieve the required weight than it did the tenderized hams. However, the difference was not as great as reported by Kemp and Fox (1985). The discrepancy probably was associated with the temperature of the hams when boned and placed in cure. In the earlier work the hams were free of ice crystals and the internal temperature was near that of the coolers (2–4°C). In this experiment, many hams contained ice crystals and thus the needle penetration did not affect juice as much.

At the time of packaging the total ham yields were similar for both groups (81.3% vs 81.0%). After holding 4 wk (Table 4) the yields had dropped less ($P<0.01$) in the packaged subgroups of both the nontenderized and tenderized hams. This showed, therefore, that packaging could reduce further weight losses while allowing aging to continue.

In order for vacuum packaging to be used, however, the hams must not have defects that are detrimental to their quality. The hams therefore were removed from vacuum packaging and examined for external appearance and internal color, aroma and general appearance. All nonpackaged hams and most packaged hams after unpackaging had normal color. A few bagged hams had a slight off odor when opened but most of the odor quickly dissipated. Subjective evaluations of color, aroma and general appearance were similar for all groups with aroma being moderately aged, color being red and general appearance being mostly excellent (Table 4). Palatability scores, except for saltiness, were similar for all groups. The mean saltiness score for the nonbagged tenderized group was higher ($P<0.05$) than for the other groups. This agreed with the higher salt and lower moisture percentages also noted in Table 4. Both tenderization and lack of packaging allowed additional moisture

Table 4—Effect of tenderizing and vacuum packaging on ham traits

	Nontenderized		*Tenderized*	
	Nonpackaged	*packaged*	*Nonpackaged*	*packaged*
Final yield	72.9[a]	79.8[b]	71.7[a]	79.5[b]
Subjective scores				
Color	2.9	2.8	2.8	3.0
Aroma	3.0	3.0	3.0	2.9
General appearance	3.9	4.0	3.9	3.7
Palatability scores				
Tenderness	5.1	5.6	5.4	5.6
Flavor intensity	5.7	5.8	5.9	5.6
Flavor preference	5.9	5.6	5.8	5.8
Saltiness	5.5[c]	5.4[c]	5.7[d]	5.7[d]
Overall satisfaction	5.8	5.6	5.7	5.7
Shear values, kg/2.5 cm cores				
SM	7.0[a]	7.1[a]	5.3[b]	5.9[b]
ST	7.1	5.6	5.5	6.7
BF	8.1[a]	9.2[b]	7.2[a]	10.1[b]
Ham composition				
H_2O, %	58.5[a]	62.1[b]	58.4[a]	62.1[b]
Salt, %	5.1[a]	4.5[b]	5.4[a]	4.8[b]
Nitrite, ppm	8.6	9.1	14.3	8.7
Microbiological counts, Log_{10}				
Total aerobic	2.68[a]	7.85[b]	4.19[a]	7.70[b]
Lactobacilli	0[a]	2.90[b]	0.17[a]	3.92[b]
Yeasts and molds	2.68	3.03	4.21	2.45

[ab] ($p<0.01$) means in rows with different superscripts are different.
[cd] ($p<0.05$) means in rows with different superscripts are differnt.

to migrate to the surface and evaporate causing a higher salt and lower moisture content especially in the tenderized nonpackaged group.

Shear values were lower ($P<0.01$) for the SM muscle in the tenderized hams with no difference due either to tenderizing or packaging for the ST muscle and higher shear values ($P<0.01$) for the packaged hams in both tenderization groups. These data appeared to be contradictory but may be partially explained by the muscle exposure. The SM muscle was more fully exposed and probably was warmer and softer at the time of tenderizing and received more muscle disruption. It is somewhat surprising, however, that the packaged BF muscles were less tender than the nonpackaged in both tenderization groups as they contained more moisture, less salt and were softer than the nonpackaged hams. Softness, however, does not necessarily mean improved shear tenderness.

Comparison data showed that the nonpackaged group contained more salt ($P<0.05$) and less moisture ($P<0.05$) within tenderization group than the packaged group. Nitrite levels were not different among groups with all being well within acceptable levels.

Tenderizing had no significant effect on either of the microbiological parameters studied. Packaging however, caused higher ($P<0.01$) aerobic and lactobacilli counts, and although higher in packaged hams, were still low enough to be of no practical significance. Yeast and mold counts were not affected by treatment and were well within expected levels (Kemp et al., 1979, 1981. 1983; Leak et al., 1984).

In general, the use of mechanical tenderization and vacuum packaging reduced curing and aging time while controlling yields of dry-cured hams.

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*MICROBIAL GROWTH ON LEAN AND SALTY TISSUE. . .*From page 8

The more rapid fall in pH of muscle from electrically stimulated carcasses could have some effect on reducing the growth of some bacteria compared to growth on non-electrically stimulated muscle whose pH fall would be slower. In view of the findings that bacterial development on lean and fat surfaces may differ considerably, the role of the exposed intramuscular fat (differences in degree or marbling) on the shelf-life of refrigerated red meat needs to be evaluated. In addition, the question may be raised as to whether microbial activities and therefore sensory characteristics and shelf-life of cuts packaged and stored with little or no surface fat would differ from comparable cuts with a normal quantity of surface fat.

In the interpretation of the results of the present study, certain limitations need to be recognized. Because samples were inoculated with single test cultures, interactions of microorganisms in mixed populations, as they occur on commercial samples, are not considered.

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Effects of Preslaughter Exercise, Electrical Stimulation, Type of Packaging Tray, Display Temperature, and Time on Acceptance and Shelflife of Beef Steaks

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ABSTRACT

Eight steers, of which four were exercised prior to slaughter, were slaughtered and one side of each carcass electrically stimulated (ES). Steaks from combined wingrib and sirloin were packaged in two types of plastic foam trays, overwrapped with PVC and displayed in refrigerated display cabinets either at 0°C or 5°C meat surface temperature. Display temperature and period of display significantly influenced shelflife and overall appearance of steaks. ES enhanced tenderness and improved muscle color but caused more weight loss during display when compared with steaks from unstimulated carcasses. All factors investigated had a significant influence on percentage weight loss during display while stress and tray depth also negatively influenced cooking weight loss encountered during cooking.

INTRODUCTION

COLOR, appearance and presentation are the principal factors which effect the sale of meat in refrigerated display cases. However, there are various factors such as animal stress, carcass electrical stimulation (ES), meat temperature and display duration that may influence the appearance and shelflife of fresh meat. Savell (1979) reported that increased tenderness is not the only advantage of electrical stimulation but that there are additional advantages, including more attractive visual appearance and longer shelflife. However, it was found by Mrigadat et al., (1980) that ES did not reduce the aerobic plate count (APC) of the skin surface or ground beef significantly. Stressed animals sometimes yield dark, firm and dry (DFD) meat, which has long been recognized to spoil more rapidly than normal meat (Somey et al., 1982; Newton and Gill, 1978). These factors (exercise, ES, display temperature and display period) in combination could have a significant influence on meat appearance and shelflife. In a review, Eustace (1979) stated that temperature had the greatest effect on the growth rate of bacteria on meat. The lag phase was extended at low temperatures, hence the retarded growth rate. With a given bacterial count on meat, the temperature of storage dictated the time to spoilage (Eustace, 1979). The appearance and presentation of the meat in the display cabinet depend on the skill of the butcher and the packaging material used. By using a deep tray (22.2 x 13.3 x 2.0 cm) it is possible to present the product in a more appealing way as the film is not in direct contact with the meat surface. It was shown by Malton (1980) that this air headspace could result in the so-called "greenhouse" effect with large temperature differences between the air and the meat, but details on shelflife were not reported.

The purpose of this study was to investigate the influence of preslaughter exercise, carcass ES, and depth of meat packaging container on overall appearance and the shelflife of displayed beef steaks.

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MATERIALS & METHODS

Exercise and electrical stimulation

A total of eight steers, 18–20 months of age, were used in this experiment. Four animals were artificially stressed by chasing them up and down a passageway between pastures for 30 min at a time, three times a day, for two days prior to slaughter and once immediately prior to slaughter. The well-rested control animals and the exercised animals were slaughtered simultaneously at the research abattoir at the Animal and Dairy Science Research Institute. The right carcass sides were electrically stimulated with a potential difference of 500 V at a frequency of 15 Hz for 2 min immediately after dressing and splitting of the carcasses, within 30 min after stunning. The four treatments therefore comprised: (a) exercised — non-ES sides; (b) exercised — ES sides; (c) control — non-ES; (d) control — ES sides.

Packaging and display of steaks

After chilling the sides for 24 hr, 1.5 cm thick steaks were cut from the boneless combined wingrib (*M. longissimus thoracis*) (11 through 13 rib section) and sirloin (*M. logissimus lumborum*) (1 through 6 lumbar vertebrae). The steak surfaces were cleaned with plastic scrapers to remove any bonedust before weighing, placed in two types of plastic foam trays, overwrapped with polivinylchloride (PVC) film and heat-sealed. Deep (Model 73D – 22.2 × 13.3 × 2.0 cm) and shallow (Model 73S – 22.2 × 13.3 × 0.9 cm) trays (Bakke Industries Ltd, S.A.) were used to determine their influence on shelflife and overall appearance of the product during display. The prepackaged meat was displayed in selfservice display cabinets, under fluorescent (warm white, color 3) lighting measuring 1050 lux at the meat surface for 24 hr per day. The cabinets were in an air-conditioned room with the ambient temperature fluctuating between 19° and 23°C. The temperature of the cabinets was adjusted to provide meat surface temperatures of 0°C or 5°C respectively in two display cabinets. Electric defrost cycles with a duration of 30 min were programmed for 06h00, 14h00, and 22h00 each day. The meat was sampled and evaluated before display and then after 3 and 5 days of display.

Bacterial plate counts

Two meat samples of known surface area (6.42 cm^2) and approximately 0.4 cm thick were cut from the central area of each steak using a sterile coring device, scalpel and forceps. The samples were homogenized in a Stomacher 400 with 100 ml sterile 1/4-strength Ringer solution (Merck). Appropriate dilutions were made, spread on nutrient agar (Merck), and incubated for 24–48 hr at 30°C. Aerobic plate counts (APC) are reported as $\log_{10}$ per cm^2.

Visual panel

Overall appearance of the meat was determined by a six-member trained panel. Meat was evaluated for overall appearance and discoloration on an eight-point scale, where one represented extremely unacceptable color and eight represented extremely acceptable color.

Taste panel and shear force

As the wholesale cut was divided into steaks, the successive steaks were paired to facilitate a valid evaluation of the taste panel, tenderness and the microbiological data on adjacent steaks undergoing the same treatment. The steaks (n = 120) were roasted in a 160°C oven to an internal temperature of 70°C. The tenderness of the meat cubes was evaluated by a trained sensory panel using a five-point scale where one represented very tough and five very tender. The panel size was restricted to four panelists due to the size and triangular shape of the steaks. Three 1.3 cm cores were cut along the grain for each

steak before taste panel samples were taken and shear-tested across the grain. Their tenderness (shear force in Newtons) was also evaluated with an Instron materials testing machine fitted with a Warner-Bratzler shearing attachment.

pH and weight measurement

A slurry was prepared by blending the meat (2g/10 mL distilled water) from each steak, after it was displayed, in an Ultra-Turrax homogenizer operated at 20,000 rpm for 1 min. The pH of this slurry was measured with a combination glass electrode. The weight of drained steaks before and after display was determined, and the difference expressed as a percentage of the initial weight. The pre- and post cooking weights were determined. The difference was expressed as a percentage of the initial weight.

Statistical evaluation

All the data were analyzed by means of a mixed model least-squares and maximum likelihood computer programs LSML76 by Harvey (1977). Although LSML76 is referred to as a mixed model least-squares program, analyses where all sources of variation (discrete and/or continuous independent variables) are fixed, are readily computed with LSM76. The data were analysed to demonstrate whether the treatments of exercise, ES, display temperature, display time and tray depth had any significant effect(s) on microbial growth, visual panel scores, taste panel scores and physical measurements. In all cases the least-squares mean was corrected for the variables not included in the particular analysis.

RESULTS

Microbiology

Temperature had a significant effect on the growth rate of organisms. At 0°C, for steaks displayed at 0, 3 and 5 days there was a mean log total plate count of 5.34 bacteria per cm^2, compared to 6.90 bacteria per cm^2 at 5°C (Table 1). Tray depth also had a significant effect on the growth of bacteria. The mean total aerobic count on steaks in shallow trays (PVC touching meat) was significantly lower than that obtained from deep trays (PVC not touching meat) (Table 1). The period of display caused a significant effect on the bacterial growth of the packaged steaks. Only the interaction between temperature and time was statistically significant, (Fig. 1). Thus, steaks displayed at the lower surface temperature would be expected to have a longer shelflife, if shelflife is defined in terms of aerobic plate counts. The mean microbial count in displays for 0, 3 or 5 days did not differ significantly between steaks from exercised vs nonexercised animals. The APC after 5 days was 1.6×10^7 per cm^2 for nonexercised and 2.3×10^7 per cm^2 for meat from exercised animals.

Overall appearance

Temperature, display period, ES and exercise were the four main effects with a significant influence on discoloration and overall appearance. From Table 2 it is evident that the lower display temperature was responsible for less discoloration and thus more acceptable meat. The period of display also had a significant influence on the discoloration and overall appearance of the displayed meat (Table 2). Electrical stimulation

Table 1—Effect of major variables (main effects on aerobic plate counts[a]

Main effect	Variables	Aerobic Plate count (Log/cm²)
Temperature	0°C	5.34**
	5°C	6.90
Tray depth	Shallow (0.9 cm)	5.87**
	Deep (2.0 cm)	6.35
Display period (days)	0	5.27a**
	3	5.77b
	5	7.30c

[a] Values in the same column having different letters are significantly different.
** P≤0.01

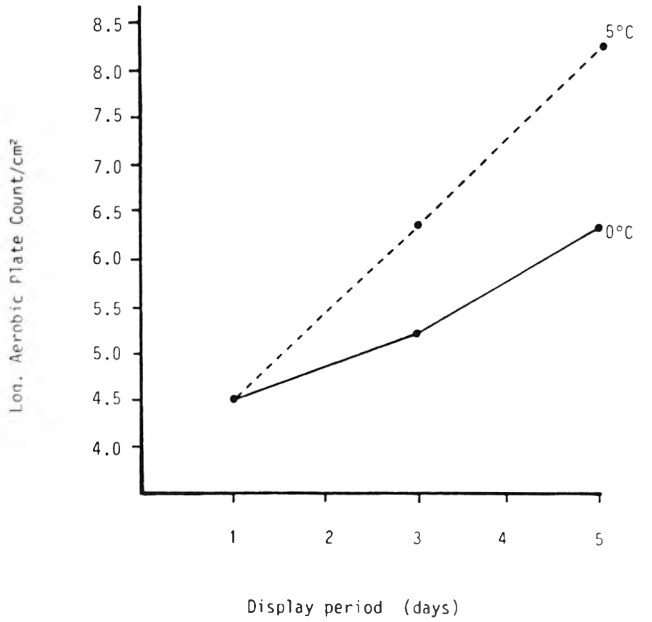

Fig. 1—Temperature-time interaction effect on the total aerobic plate count.

Table 2—Effect of temperature, display period, electrical stimulation (ES) and exercise on discoloration and overall appearance of the displayed steaks[a]

Main effects		Discoloration[b]	Overall appearance[c]
Temperature	0°C	6.46**	4.76**
	5°C	4.85	3.06
Display period (days)	0	6.39a**	5.15a**
	3	5.87ab	3.55b
	5	4.70b	3.02c
ES	ES	6.08**	4.10[NS]
	Control	5.23	3.72
Exercise	DFD[d]	5.41[NS]	3.71[NS]
	Control	5.90	4.10

[a] Values in the same column having different letters are significantly different.
[b] Discoloration: 1 = completely discolored; 8 = extremely acceptable color.
[c] Appearance: 1 = extremely unacceptable; 8 = extremely acceptable color.
[d] DFD = Dark, firm and dry.
**P≤0.01; NS = Nonsignificant

significantly affected the discoloration of the steaks ($P \leq 0.01$) with ES-steaks having a higher color score than steaks from non-ES sides.

The interaction between temperature and time had a significant effect on panel acceptability. Initially, the panel rated the meat as acceptable (6.04; Fig. 2) with a discoloration score of 7.25 (Fig. 3). At a 5°C display temperature these values dropped to 1.82 and 3.40, respectively, after a 5-day display period, compared to 4.22 and 6.00, respectively, at a meat surface display temperature of 0°C. Overall appearance value differences were significant at the 1% and those for discoloration at the 5% level of confidence. Steaks from control animals had a mean pH-value of 5.6 while the mean pH for meat from exercised animals was 5.9 (Fig. 4).

Taste panel

Steaks from ES sides were significantly ($P \leq 0,01$) more tender than control steaks (Table 3). Furthermore, it was found, that there was a progressive tenderizing tendency due to the time of display. Meat from the ES-sides was more tender than from the control sides, but exercise prior to slaughtering resulted in a diminished ES-tenderizing effect. Instron shear tests also showed the ES steaks were more tender than controls. A sim-

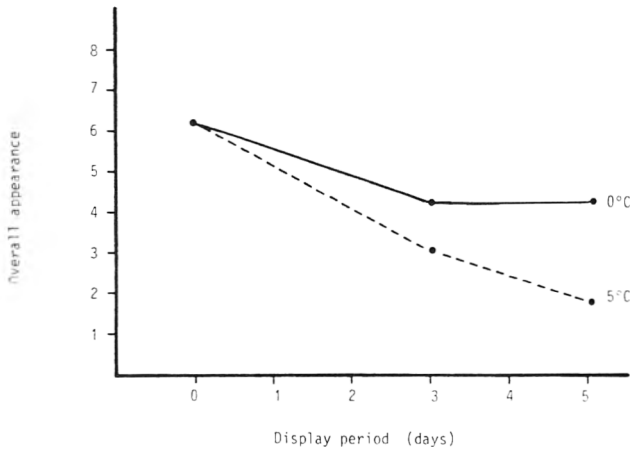

Fig. 2—Temperature-time interaction effect on the overall appearance of steaks judged by a trained panel: 1 = extremely unacceptable; 8 = extremely acceptable.

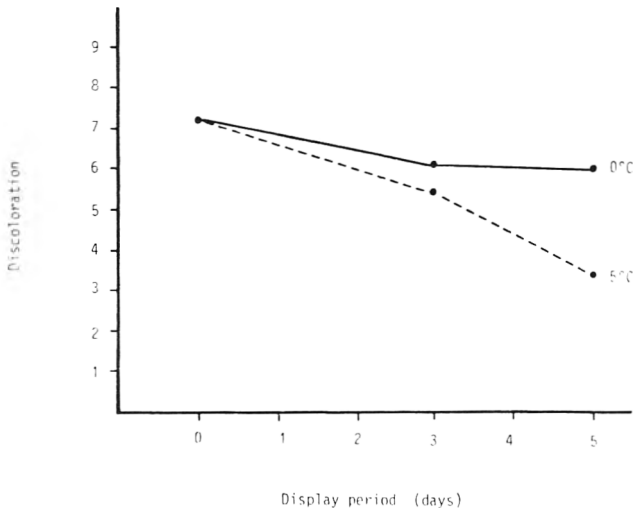

Fig. 3—Temperature-time interaction effect on the discoloration of steaks judged by a trained panel: 1 = completely discolored; 9 = no discoloration.

ilar, but smaller statistically significantly tenderizing effect due to aging is also shown by measuring the shear values at 0, 3, and 5 day intervals ($P \leq 0.05$).

Steak weight loss

At the higher temperature (5°C) a higher weight loss (1.23%) occurred ($P \leq 0.01$), (Table 4). In addition, display time contributed significantly ($P \leq 0.01$) to the weight loss during display. Steaks packaged in deep trays lost more weight ($P \leq 0.01$) during display than those packaged in shallow trays.

Cooking weight loss

Exercise and tray depth had significant effects on the cooking loss during oven roasting of the cuts (Table 5). The control steaks lost approximately 1.6% ($P \leq 0.05$) more weight than steaks from exercised animals and steaks that had been packaged in shallow trays lost approximately 2.0% ($P \leq 0.01$) more weight than steaks in deep trays.

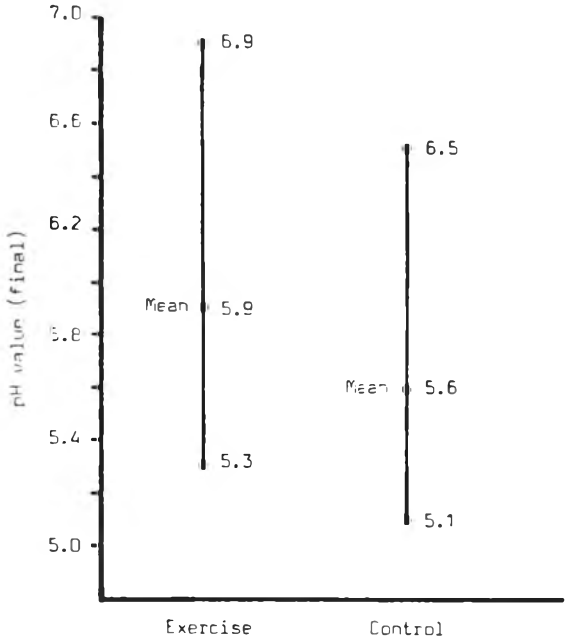

Fig. 4—Final pH values of steaks from exercised and control animals.

Table 3—Effects of electrical stimulation (ES), display period and side treatment on sensory tenderness and shear value (Newtons)[a]

Main effect	Variable	Tenderness[b]	Shear value (Newton)
ES	ES	3.07**	65.36**
	Control	1.77	87.13
Display period (days)	0	2.16a**	82.61a*
	3	2.41b	74.68ab
	5	2.69c	71.46b

[a] Values in the same column having different letters are significantly different.
[b] 1 = very tough; 5 = very tender
*$P \leq 0.05$
**$P \leq 0.01$

Table 4—Effect of exercise, display temperature, display period and tray type on the steak weight loss

Main effect	Variable	Package wt loss (%)
Temperature	0°C	2.29**
	5°C	3.52
Display period (days)	3	1.85**
	5	3.95
Tray	Shallow (0.9 cm)	2.71**
	Deep (2.0 cm)	3.08

**$P \leq 0.01$

Table 5—Effect of stress and tray depth on the cooking weight loss

Main effect	Variable	Cooking weight loss (%)
Exercise	Exercise	17.10*
	Control	18.73
Tray	Shallow	18.93**
	Deep	16.90

*$P \leq 0.05$
**$P \leq 0.01$

DISCUSSION

THE OBSERVATION that lower counts were obtained at a lower temperature is in agreement with the finding of Eustace (1979) that among the various factors which influence the growth rate of bacteria on meat such as temperature, moisture, nu-

trients and pH, temperature has the greatest effect on the growth rate of bacteria on meat.

Lower counts were obtained from steaks in shallow trays, where the film is in close contact with the meat surface. Malton (1980) showed that meat surface temperature was close to the ambient air temperature in display cabinets only when the product was tightly wrapped. A "greenhouse" effect in the trays overwrapped with film was clearly demonstrated (Malton, 1980) by a large temperature difference between the air and the meat. The present study supports the findings of Malton (1980) and illustrates the effect of the higher temperature prevalent in the film overwrapped trays on the bacterial growth that occurs during display, in comparison with trays where the film is in close contact with the meat surface. The present data illustrate the significant effect of temperature on bacterial growth as indicated by Eustace (1979).

Results concerning the effect of ES on microbial counts are in accordance with previous findings in the literature (Mrigadat et al., 1980; Nottingham, 1982), that there are no significant differences in the shelflife of electrically stimulated versus unstimulated meat.

Although it has been observed (Nottingham, 1982) that meat from exercised animals (DFD-meat) spoils more rapidly than normal meat, this could not be proved conclusively in the present study. In DFD-meat, glycogen is depleted or absent, thus spoilage bacteria have to utilize amino acids at an earlier stage of growth than on normal meat. As spoilage odors and flavors are the result of amino acid degradation, they might therefore become detectable at lower bacterial cell densities (Gill, 1982). The fact that differences obtained in this study were not statistically significant could be attributed to the fact that some non-exercised animals were very stress susceptible and, as will be discussed later, had DFD-meat with a high pH.

Factors affecting color changes on meat surfaces, listed by Lanier et al., (1977); include: (1) partial pressure of oxygen in the environment, (2) bacterial growth, (3) artificial atmospheres, (4) temperature, (5) tissue lipid oxidation, and (6) drying of the meat surface.

Temperature plays an important role in determining the growth rate of bacteria on meat surfaces as lag times are increased and growth rates are decreased by cooling (Eustace, 1979). The storage temperature will largely dictate the spoilage time. However, no bacterial activity is necessary for pigment oxidation under low oxygen pressure, and the primary role of bacteria in meat discoloration is the reduction of oxygen tension in surface tissue (Walker, 1980). The effect of temperature and time on overall appearance and discoloration can thus be attributed, in part, to the growth of organisms on the meat surface.

Steaks from ES sides had a more acceptable color than steaks from control sides, not withstanding tray depths. Research carried out at the Texas Agricultural Experiment Station indicated that increased tenderness is not the only advantage of ES, but that additional benefits, are better flavor, more attractive visual appearance and longer shelflife (Savell, 1979; Cross, 1979).

Steaks from the nonexercised animals generally had lower mean final pH-values than those from exercised animals. Stressed animals had some pH-values lower than 5.95 and had normal meat. The significant differences in pH-values support the finding of color differences between DFD and normal meat. The higher pH in DFD-meat contributes to the dark, firm and dry characteristics of the meat and also enhances the growth of organisms which results in a shorter shelflife and consumer discrimination (Newton and Gill, 1978, 1980).

In the present study there was an advantage of increased tenderness due to electrical stimulation which is in accordance with findings by various authors (Savell et al., 1978: Cross, 1979; Savell, 1979; Mrigadat et al., 1980). It was also shown than tenderness is improved and that additional aging occurs in the display cabinet after retail packaging of steaks.

It is also obvious from this study that both the higher storage temperature and longer display time resulted in increased weight loss. This effect is ascribed to the higher rate of evaporation.

Steaks in deeper trays had statistically significant higher weight loss than those in the shallow trays which is attributed to the "greenhouse" effect. According to Malton (1980) large temperature differences occurred between the air immediately surrounding the meat and the meat itself in the film overwrapped trays ($-1°$ vs 6.2°C). This higher (7.2°C) temperature brought about a higher rate of evaporation resulting in the greater weight loss in these packs.

The low cooking loss of meat from exercised animals probably results from the high ultimate muscle pH, which can have practical advantages. In its fresh form, this meat is also subject to a lower weight loss during storage and cooking than meat with a lower ultimate pH. (Sorney et al., 1982). Steaks packaged in deep trays lost more weight during display due to moisture evaporation and hence had less to lose during cooking. This result accentuated the influence of the "greenhouse" effect on weight loss during chilled display.

CONCLUSIONS

IN SUMMARY, it appears that low display temperatures and the length of the display period had a beneficial influence on the shelflife, overall appearance of retail steaks, and on the weight loss that occurred during display, Electrical stimulation enhanced the tenderness and improves muscle color. The shorter display period at low case temperature had the biggest positive effect on the percentage weight loss during display, although shallow trays also had an advantageous influence on aerobic plate counts, package weight loss and cooking weight loss.

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Antimicrobial Activity and Functionality of Reduced Sodium Chloride and Potassium Sorbate in Uncured Poultry Products

JOHN N. SOFOS

ABSTRACT

Uncured turkey and chicken breast meat products, formulated with NaCl (0.65 and 1.30%) and potassium sorbate (0 and 0.26%), were tested for antimicrobial activity and functional properties. The chopped products were inoculated (*Clostridium sporogenes*, 10/g) in cans and in packages and abused at 20°C. Microbial growth and gas production were rapid at both NaCl levels tested. Inclusion of potassium sorbate in the formulation delayed initiation and rate of microbial growth and gas production. Potassium sorbate also reduced ($P<0.05$) weight losses during cooking of the low NaCl turkey product. Rates of gas production were similar in canned and vacuum packaged products. Differences in microbial growth and gas production between chicken and turkey products were minor.

INTRODUCTION

THE DISTRIBUTION and sales of cooked, uncured, vacuum-packaged poultry products have increased in recent years. These products contain poultry meat, salt and polyphosphates, and are pasteurized to a temperature of about 70°C. After thermal processing, the products are usually sliced, vacuum packaged and sold in the refrigerated case. A new system of processing ("cook-in") has been developed in recent years and involves cooking and marketing of these products in the same plastic pouch (Siegel, 1982). The microbial stability of these products, which do not involve post-process contamination, is largely unknown.

Uncured poultry products of the above types contain salt levels lower than traditional cured meat products. This, coupled with the absence of nitrite and the fact that the products are pasteurized and vacuum packaged, makes them suitable media for the growth of clostridia and other microorganisms in case of abuse. Thus, there is a need for research to study the microbial stability of these products.

The possible role of dietary sodium in the development of hypertension in certain individuals has prompted public health and regulatory authorities to recommend reducing dietary intakes of sodium chloride (Anonymous, 1980). As NaCl levels are reduced, however, meat product binding and water holding capacity, as well as preservative capacity are decreased (Trout and Schmidt, 1984; Sofos, 1983, 1984). Partial replacers of sodium chloride suggested in meat products include polyphosphates and other chloride salts (Madril and Sofos, 1985; Sofos, 1984).

Work with red meat and mechanically deboned poultry products has demonstrated that sorbic acid or potassium sorbate are effective inhibitors of microbial growth (Sofos et al., 1979; Sofos and Busta, 1980, 1981; Robach and Sofos, 1982). Studies on the antimicrobial activity of sorbate in uncured, luncheon-type poultry products are limited. Robach et al. (1980) and To and Robach (1980) have reported on the potential of *Salmonella* sp., *Staphylococcus aureus* and enteropathogenic *Escherichia coli* to grow in these products. It was also indicated that potassium sorbate and sorbic acid suppressed growth of these pathogens and extended product shelf-life. Studies by Huhtanen and Feinberg (1980) indicated that potassium sorbate and sorbic acid were influential in delaying *Clostridium botulinum* growth in chicken and turkey frankfurters formulated with no nitrite. The results also indicated a difference in sorbate effectiveness between turkey and chicken formulations, with turkey being affected the most and sorbic acid being more effective than potassium sorbate.

Since published research on the shelf-life and safety of uncured, turkey breast meat and chicken products formulated with varying levels of salt in almost nonexistent, the objectives of this study were to determine microbial growth and shelf-life (20°C) of such products formulated with varying sodium chloride and potassium sorbate levels and inoculated with *Clostridium sporogenes* spores. Additional objectives included comparison of antimicrobial activity in chicken and turkey products and determination of the influence of inoculating before and after thermal processing on product shelf-life.

MATERIALS & METHODS

Processing and inoculation

Uncured, pasteurized chicken and turkey breast meat products were formulated with NaCl (0.65%, 1.30%), potassium sorbate (0%, 0.26%), sodium tripolyphosphate (0.4%) and ice (10%) (potassium sorbate and sodium tripolyphosphate were provided by Monsanto Company, St. Louis, MO). The meats (5 kg batches) were hand-deboned broiler chicken and young turkey breasts (H. W. Longacre Co., Franconia, PA and Longmont Foods, Longmont, CO). They were mixed with the other ingredients and coarse chopped in a Meissner model VE chopper (RMF Steel, Kansas City, MO) at low bowl speed and with two blades (2,000 rpm blade speed). The products were then extruded into 303 × 406 cans (9/treatment) provided by American Can Company (Chicago, IL) with an E-Z Pak water-powered piston stuffer.

Five cans per treatment were inoculated with a heat-shocked (80°C, 15 min) suspension of *C. sporogenes* P.A. 3679 spores. The stock culture was provided by the National Food Processors Association and sporulated in a trypticase medium according to standard procedures. Inoculation was accomplished by introducing 10 mL of spore suspension with a syringe into each can. The target inoculum level was 10 spores per gram of product or approximately 5,000 spores per can. Inoculated and uninoculated cans were sealed with a Dixie can closing machine and heat-processed to a final internal product temperature of 70°C in an open, air-agitated and thermostatically controlled retort (Dixie Equipment Company, Athens, GA) at a water temperature of 80°C. The total heat processing time was 75 min. 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 the treatment cans. After thermal processing the cans were water (6–8°C) cooled and stored overnight in a 2°C cold room.

Part of the product from the uninoculated cans was sliced (after thermal processing), and the slices were inoculated with heat-shocked (80°C, 15 min) *C. sporogenes* spores. The target inoculum level was 10 spores per gram which was spread as a 0.5 mL suspension between three slices. Packages (20/treatment) with three slices of inoculated product in each were vacuum-sealed and stored together with the inoculated cans for abuse at 20°C.

Testing

The product from the uninoculated cans was also used to determine losses during thermal processing, force (kg) needed to shear the prod-

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Table 1—Weight losses (%) during thermal processing and force needed to shear cooked, uncured turkey and chicken breast meat products formulated with varying levels of NaCl (0.65 and 1.30%) and potassium sorbate (0 and 0.26%)

	Turkey				Chicken			
NaCl (%)	0.65	1.30	0.65	1.30	0.65	1.30	0.65	1.30
Sorbate (%)	0	0	0.26	0.26	0	0	0.26	0.26
Losses (wt. %)	19.13[a]	1.51[b]	4.51[b]	1.30[b]	1.08[b]	0.45[b]	0.84[b]	0.97[b]
Shear force (kg)	1.36[a]	1.32[a]	1.51[a]	1.71[b]	0.59[c]	0.76[cd]	0.74[cd]	0.88[d]

[a-d] Means in the same row with at least one superscript the same were not significant (P>0.05).

Table 2—Composition of cooked, uncured turkey and chicken breast meat products formulated with varying levels of NaCl (0.65 and 1.30%) and potassium sorbate (0 and 0.26%)

	Turkey				Chicken			
NaCl (%)	0.65	1.30	0.65	1.30	0.65	1.30	0.65	1.30
Sorbate (%)	0	0	0.26	0.26	0	0	0.26	0.26
Fat	1.38[a]	1.24[ab]	1.04[b]	1.03[b]	3.47[c]	3.32[c]	3.99[d]	3.35[c]
Moisture	72.17[a]	72.50[a]	73.11[b]	74.13[c]	74.04[c]	73.06[b]	72.39[a]	73.73[c]
Brine[d]	0.90[a]	1.78[b]	0.89[a]	1.74[b]	0.88[a]	1.77[b]	0.90[a]	1.75[b]

[a-c] Means in the same row with at least one superscript the same were not significant (P>0.05).
[d] NaCl in the water phase of the product (% wt/vol).

uct and fat, moisture and NaCl levels. Cook losses were determined by opening the uninoculated cans and removing and weighing the material that had separated. The loss was expressed as percent of the raw product weight.

Shear force (kg) was measured with a modified Warner-Bratzler meat shear, model 2000 (G. R. Electric Manufacturing Company, Manhattan, KS) with a single blade. The dial spring balance was replaced with a load cell and an amplifier which was connected to a strip chart recorder to provide more accurate readings (Sofos, 1983; Trout and Schmidt, 1984). Five room temperature sample plugs, 2 cm in diameter and 2 cm in length, were sheared per treatment.

Product moisture, fat and NaCl were determine according to standard procedures (AOAC, 1980). Initial pH values and pH changes during storage of vacuum-packaged products were determined on 30-g samples blended with 270 mL sterile peptone (0.1%) diluent which was also used for microbiological analyses. A Corning calomel electrode in conjunction with a Corning model 125 pH meter was used for pH determinations.

Microbiological evaluation

Inoculated cans and vacuum packages were stored at 20°C and monitored daily for visual gas production (swelling). When gas was detected, the product was checked for putrefaction. Microbial counts determined in inoculated packages during 20°C storage included total mesophilic anaerobic counts, mesophilic anaerobic spore (80°C, 15 min) counts and total aerobic mesophilic counts. Microbial analyses (and pH measurements) were performed by aseptically blending 30g of sample with 270 mL sterile peptone (0.1%) diluent. Serial dilutions were made in sterile 0.1% peptone water and used to inoculate Lee tubes (Ogg et al., 1979) for total mesophilic anaerobic counts and spore counts (24–48 hr, 37°C) and petri plates with APT agar for total aerobic counts (48 hr, 37°C). Anaerobic counts were determined in trypticase soy agar (BBL) modified by the addition of 0.1% dextrose, 0.05% cysteine-HCl and 0.1% resazurin as an indicator of reduced conditions. Before inoculation, 0.2 mL of a filter-sterilized solution of $NaHCO_3$ (86 mg/mL) were added in each Lee tube (Foegeding and Busta, 1983).

Statistical Analysis

The experimental design was a complete 2 × 2 × 2 factorial [2 meats (chicken, turkey) × 2 NaCl levels (0.65%, 1.30%) × 2 potassium sorbate levels (0%, 0.26%)] with two replicates conducted on two different occasions. The data were analyzed by analysis of variance and when the F values were significant, Fischer's Least Significant Difference (LSD) was used to separate statistical differences between treatment means. The gas production data from the two replicates were combined by a computer program which gave percentage of cans and packages showing gas at specified time intervals during product storage.

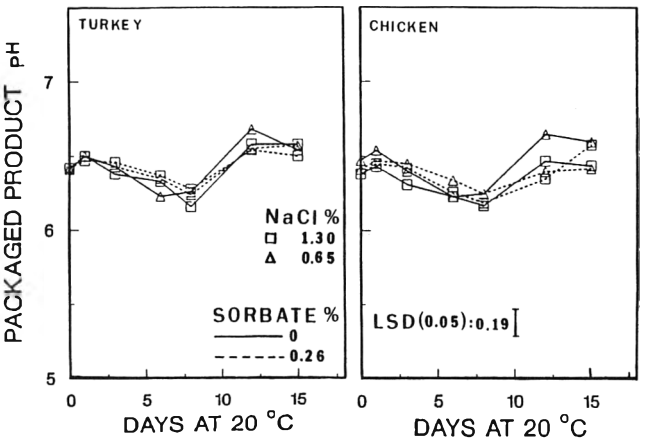

Fig. 1—Changes in pH during storage (20°C) of cooked, uncured turkey and chicken breast meat products inoculated with Clostridium sporogenes *spores (10/g) after thermal processing (70°C) and before vacuum packaging (day 0, raw product; day one, cooked product; two replicates).*

RESULTS & DISCUSSION

Processing losses

As the amount of NaCl (Table 1) was reduced in the turkey breast formulation, weight losses (%) during thermal processing increased ($P<0.05$). The high weight loss (19.13%) of the turkey product with low (0.65%) NaCl and no sorbate (Table 1) did not appear to be coincidental or due to experimental error. This is supported by the fact that high weight losses occurred in both replicates of the same treatment (16.82% in the first replicate and 21.44% in the second replicate). No explanation is readily available, however, for these high losses compared to losses in product formulated with chicken meat. Since the material separated during thermal processing included a mixture of components (water, fat, solids), the shear force (Table 1) and the proximate composition (Table 2) of the product were similar with other treatments.

Cook losses in chicken products were generally lower than those in turkey products. It was unexpected, however, to find that inclusion of potassium sorbate (0.26%) in the formulation reduced weight losses during thermal processing (Table 1). This unexpected but significant finding indicated that sorbate may be influential in improving water and other ingredient

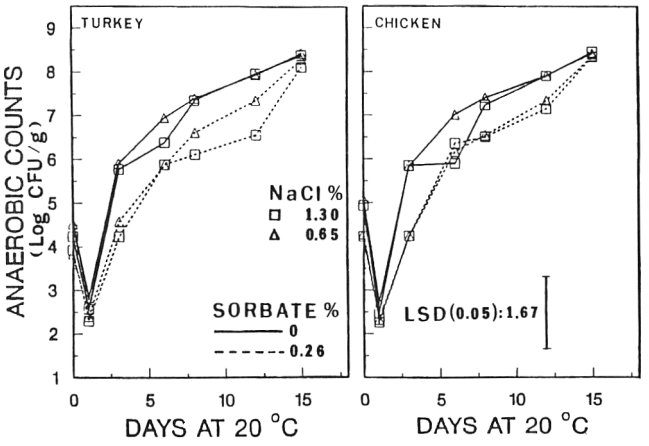

Fig. 2—Mesophilic anaerobic counts during storage (20°C) of cooked, uncured turkey and chicken breast meat products inoculated with Clostridium sporogenes *spores (10/g) after thermal processing (70°C) and before vacuum packaging (day 0, raw product; day one, cooked product; two replicates).*

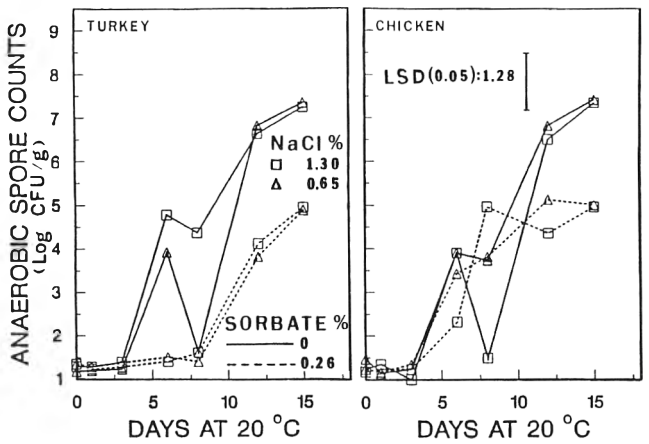

Fig. 3—Mesophilic anaerobic spore (80°C, 15 min) counts during storage (20°C) of cooked, uncured turkey and chicken breast meat products inoculated with Clostridium sporogenes *spores (10/g) after thermal processing (70°C) and before vacuum packaging (day 0, raw product; day one, cooked product; two replicates).*

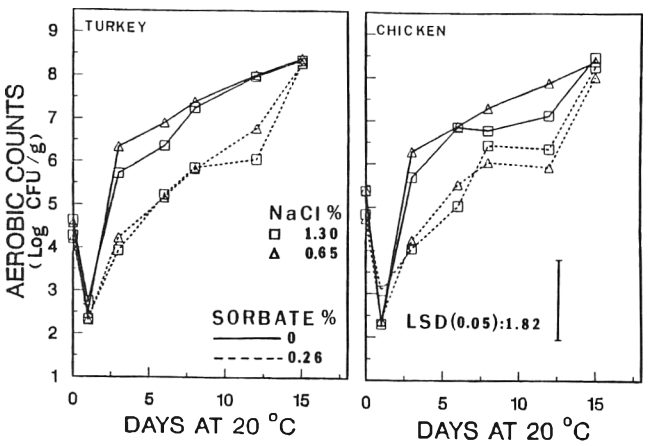

Fig. 4—Mesophilic aerobic counts during storage (20°C) of cooked, uncured turkey and chicken breast meat products inoculated with Clostridium sporogenes *spores (10/g) after thermal processing (70°C) and before vacuum packaging (day 0, raw product; day one, cooked product; two replicates).*

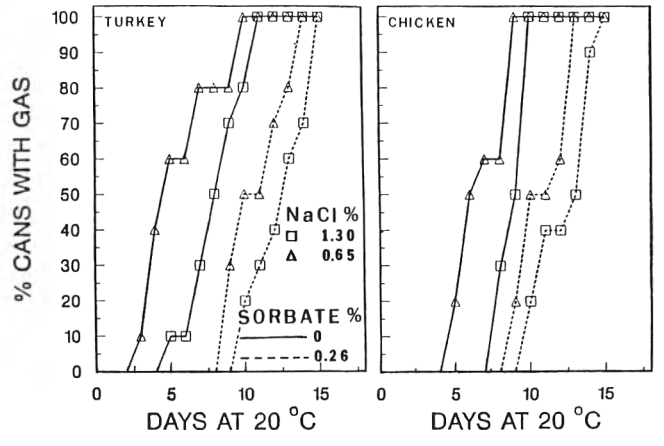

Fig. 5—Rate and extent of gas production during storage (20°C) of canned, cooked, uncured turkey and chicken breast meat products inoculated with Clostridium sporogenes *spores (10/g) before thermal (70°C) processing (two replicates).*

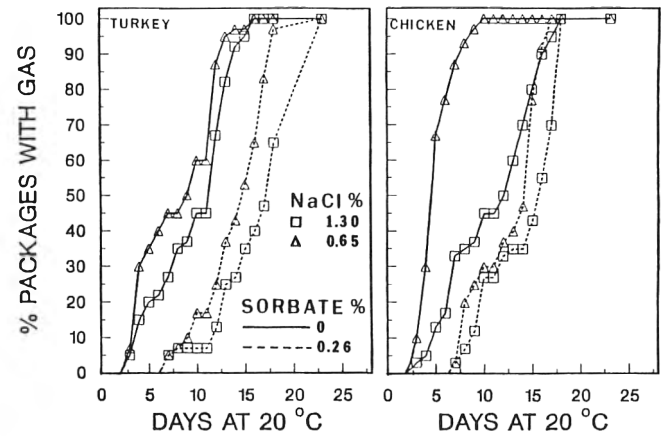

Fig. 6—Rate and extent of gas production during storage (20°C) of vacuum packaged, cooked, uncured turkey and chicken breast meat products inoculated with Clostridium sporogenes *spores (10/g) after thermal (70°C) processing (two replicates).*

retention in meat products. This influence of sorbate was observed in both replicate experiments. The high losses in the low NaCl turkey product were comprised of both fluid and solids.

Since sorbate is only known for its antimicrobial properties, a recent study (Sofos, 1985) tested this observation is comminuted red meat (beef-pork) formulations with varying levels of NaCl. The results have confirmed the above finding and have demonstrated clearly that sorbate reduced weight losses during thermal processing of comminuted meat products formulated with varying levels of NaCl.

Product texture

The major observation in the shear force (kg) data (Table 1) is that broiler chicken breast products were significantly ($P<0.05$) softer than young turkey breast products. This result was expected, since broiler chicken meat is softer in texture than most other chicken and turkey meats. No major differences were found in product shear force with reduced brine levels. Potassium sorbate to some degree increased the shear force of both turkey and chicken products.

Product composition

Proximate analysis of the various products showed no major differences among treatments in moisture and fat (Table 2). Products with chicken meat, however, were higher in fat than

Table 3—Effects of NaCl (0.65 and 1.30%) and potassium sorbate (0 and 0.26%) on average days to first gas detection in cooked, uncured turkey and chicken breast meat products inoculated with Clostridium sporogenes *spores (10/g) in cans (before thermal processing, 70°C) and in vacuum packages (after thermal processing, 70°C) and stored at 20°C*

	Turkey				Chicken			
NaCl (%)	0.65	1.30	0.65	1.30	0.65	1.30	0.65	1.30
Sorbate (%)	0	0	0.26	0.26	0	0	0.26	0.26
Cans	4.0[a]	7.5[b]	10.5[cd]	11.5[d]	5.0[a]	9.0[bc]	10.5[cd]	12.0[d]
Packages	5.0[a]	7.5[ab]	10.0[b]	10.5[b]	3.5[a]	8.0[ab]	9.5[ab]	11.0[b]

[a-d] Means in the same row with at least one superscript the same were not significant ($P>0.05$). No significant differences existed between cans and packages of a given treatment.

products formulated with turkey meat. Brine levels (% NaCl, wt/vol) were 1.74–1.78% for the higher NaCl (1.30%) products and 0.88–0.90% for the lower NaCl (0.65%) products. These results indicated that products of acceptable composition were produced, and comparisons among treatments were feasible.

Product pH

Initial pH values (6.5) for all treatments were similar and typical for products of this type (Fig. 1). No major differences were found in initial pH values for treatments formulated with or without potassium sorbate. This permitted comparison of microbial growth in treatments formulated with or without the preservative. Potassium sorbate was preferred over sorbic acid to avoid the influence of the acid on product pH (Sofos, 1981). With storage at 20°C, pH values decreased. These decreases in pH were more rapid in treatments without potassium sorbate and coincided with development of microbial growth and product spoilage. As spoilage progressed, pH values increased.

Product shelf-life

Microbial growth. Total mesophilic anaerobic counts, anaerobic spore counts and aerobic counts during storage at 20°C are presented in Fig. 2, 3, and 4. No major differences were observed in rate and extent of microbial growth between treatments formulated with turkey or chicken meat. Both NaCl levels (1.7 and 0.9% brine) were too low to delay microbial growth. At both NaCl levels and in the absence of sorbate, anaerobic and aerobic counts increased very rapidly and reached levels of 10^6 CFU/g in 3 days of storage and 10^7 CFU/g in 6 days at 20°C. These results demonstrated that these formulations of uncured poultry breast meat products contained NaCl levels that were insufficient to delay microbial growth, assure adequate shelf-life and possible product safety in case of abuse.

Inclusion of potassium sorbate (0.26%) in the formulation resulted in reduced rates of microbial growth (Fig. 2, 3, and 4). Anaerobic and aerobic counts in the presence of sorbate were only 10^4 CFU/g after 3 days of storage and 10^5–10^6 CFU/g after 6 days of abuse at 20°C. It is interesting to note that potassium sorbate was almost equally effective in delaying microbial growth at both levels of NaCl (1.30 and 0.65%) tested. Therefore, rapid microbial growth in pasteurized uncured, chicken and turkey breast meat products containing low levels of NaCl can be delayed by inclusion of potassium sorbate (0.26%) in the formulation.

Rate of gas production. Production of gas in inoculated cans and packages during storage at 20°C is shown in Fig. 5 and 6. As the data demonstrated, rate of gas production was slower at the 1.30% NaCl level compared to the 0.65% NaCl level. Similar to its effect on microbial growth, inclusion of potassium sorbate in the formulation resulted in a delay of gas production which was proportional to the NaCl level in the formulation.

No major differences were apparent in rate of gas production between treatments formulated with turkey or chicken meat, and overall, rate of gas production appeared to be only slightly more rapid in the vacuum-packaged products compared to canned products. This difference may reflect absence of heat × spore × additive interaction in the vacuum packaged products which involved inoculation and repackaging after thermal processing.

In general, however, these data, together with data on microbial growth presented in previous paragraphs, have demonstrated that potassium sorbate was effective in improving the shelf-life of uncured, chicken and turkey breast meat products formulated with regular and reduced NaCl levels and contaminated both before and after thermal processing. The results from products in vacuum packages would reflect the situation of a product which has been sliced and repackaged after processing but before distribution. The results from canned products may be indicative of products processed under the new "cook-in" concept which does not involve opening and recontamination after thermal processing (Siegel, 1982).

Days to first gas detection. In general, detection of first gas was usually first in packaged products inoculated after thermal processing. Differences, however, between cans and packages were not significant (Table 3). Comparing days for detection of first gas between turkey and chicken products (Table 3), the results indicated similar responses between the two products. These data also demonstrated the influence of potassium sorbate in delaying gas production in all treatments formulated with the two NaCl levels, the two poultry meats (chicken and turkey), and inoculation before and after thermal processing (cans and packages).

CONCLUSIONS

THE RESULTS of this study indicated that microbial growth and gas production occurred rapidly even in the uncured products with 1.30% NaCl. This demonstrated a potential shelf-life and safety problem for these products. Spoilage, however, was slightly more rapid when the NaCl was reduced by 50% (0.65%).

Inclusion of potassium sorbate (0.26%) in the formulation significantly delayed microbial growth and gas production at both NaCl levels tested, indicating that sorbate could improve the antimicrobial properties of these sensitive products. Since gas production was delayed for double the time in the low NaCl-sorbate treatment, compared to the low NaCl level tested alone, stable uncured poultry products can be formed with very low NaCl (0.65%) levels when sorbate is included in the formulation.

The increased weight losses that occurred during cooking of the low NaCl turkey product were significantly reduced by inclusion of potassium sorbate in the formulation. This finding is significant and has been confirmed with other meat products. However, it needs further testing because it is the first indication that sorbate may be contributing to functional properties in meat products in addition to its influence as an antimicrobial agent.

Gas production rates were similar between products inoculated in cans before thermal processing and products inoculated after thermal processing and stored under vacuum in plastic pouches. Differences in microbial growth and spoilage between chicken and turkey products were not statistically significant.

Overall the results indicated that potassium sorbate can be a useful ingredient in extending the shelf-life of these sensitive, uncured poultry products. In addition, sorbate may be contrib-

—Continued on page 23

Antioxidant Property in Ginger Rhizome and Its Application to Meat Products

Y. B. LEE, Y. S. KIM, and C. R. ASHMORE

ABSTRACT

Some characteristics of an antioxidant factor in ginger rhizome were studied. The antioxidant property was evenly distributed across the cross-section of rhizome, and was very effective against lipid oxidation. Two thirds of the original activity remained after 2 hr of boiling. Its effectiveness was dependent on pH, increasing with increasing pH between 5 and 7. A linear relationship was observed between the amount of ginger extract added to the oxidation system and the protection factor values. The antioxidative effectiveness of ginger extract was further tested with fresh, frozen and precooked pork patties. The shelf life of all products determined by TBA value was improved by the inclusion of ginger extract.

INTRODUCTION

A MAJOR CAUSE of muscle food deterioration is oxidative rancidity and the subject has been extensively reviewed recently (Melton, 1983; Love, 1983; Dawson and Gartner, 1983). This oxidative deterioration of muscle involves the oxidation of the unsaturated fatty acids catalyzed by hemoproteins as well as by nonheme iron (Younathan and Watts, 1959; Liu, 1970b; Sato and Hegarty, 1971). The development of so-called warmed-over flavor in cooked meat has assumed much greater significance in recent years due to the rapid increase of precooked meat in fast foodservice facilities.

Synthetic and natural antioxidants have been utilized to retard the development of rancidity. Since the use of synthetic antioxidants is prohibited in many meat products, the effects of a variety of plant and spice extracts on the prevention of rancidity have been investigated (Watts, 1962; Pratt and Watts, 1964; MacNeil et al., 1973). Ginger rhizome has been one of the most popular spices in oriental cuisine. Several investigators in Japan reported that ginger or ginger extract added to lard or other foods showed reasonably strong antioxidant property (Kihara and Inoue, 1962; Fujio et al., 1969; Saito et al., 1976). Yet, little information is available on the characteristics of antioxidant substances present in ginger rhizome.

The objective of the present study was to investigate the properties of antioxidant substances in ginger rhizome and their effectiveness as a source of antioxidant in fresh, frozen or precooked pork patties.

MATERIALS & METHODS

Ginger extract

Fresh ginger rhizome was purchased from a local supermarket. The rhizome was peeled, sliced, ground in a mortar with pestle, and squeezed through four layers of cheesecloth to produce a crude ginger extract. The yield of crude extract was approximately 50% of the original rhizome weight. A portion of the crude extract was centrifuged at 20,000 × *g* for 20 min to separate the supernatant from the white and grainy precipitate. Other portions of extract were heated for 0, 10, 30, 60 and 120 min in a boiling water bath. Depending on the experiments, one of the above preparations was used.

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Table 1—Design and formulation of experimental pork patties

Treatments	Ginger extract (%)	Formulation: Meat (kg)	Salt (g)	Ginger extract (mL)	Water (mL)
T_1	0	5	90	0	25
T_2	0.05	5	90	2.5	22.5
T_3	0.1	5	90	5.0	20
T_4	0.25	5	90	12.5	12.5
T_5	0.5	5	90	25	0

Linoleic acid emulsion

Emulsions of linoleic acid were used as substrates for the oxygen uptake study and were always prepared immediately before use. Linoleic acid (1.5 g) was added to 6 mL 0.1M sodium phosphate buffer (pH 7.0) containing 2.5g Tween 20. The emulsification was accomplished by blending with a Polytron for 30 sec at 25°C.

Oxidation studies

A modified form of the rapid oxygen uptake method described by Berner et al. (1974) was employed. A Gilson oxygraph connected to a constant temperature water bath was used to measure the oxygen uptake. In a typical experiment, 1g linoleic acid emulsion was added to 25 mL 0.1M sodium phosphate buffer (held at 25°C in a water bath). The contents were thoroughly stirred until a homogeneous solution was obtained. A 1.2 mL aliquot of the buffered emulsion and 0.01 mL diluted ginger extract were placed in the sample vessel of the oxygraph equipped with a magnetic stirrer and an oxygen electrode. After equilibration, 0.1 mL metmyoglobin solution (12 mg/10 mL) was added and immediately the oxygen uptake was recorded. The length of induction period was measured to calculate a PF (protection factor) value according to the method described by Pokorny (1971). The effectiveness of antioxidant in ginger rhizome at various conditions was expressed in terms of PF value.

Formulation of pork patties and storage test

A side of pork carcass was deboned. All of the muscle and a portion of fat tissue were ground through 6.4 and 4.8 mm plates, mixed and divided into five different treatment batches. They were then blended with salt and various levels of crude ginger extract as shown in Table 1, followed by final grinding through a 4.8 mm plate and formation into patties of 10 cm in diameter and 1 cm in thickness. The fat content of pork patties was approximately 25%.

Thirty patties of each treatment were placed on plastic foam trays, wrapped with Saran wrap and placed in a refrigerator at 4°C. The change of meat color was observed visually and 2-thiobarbituric acid (TBA) test was performed at 0, 3, 6, 9, and 13 days.

Another 30 patties of each treatment were also placed on plastic foam trays, wrapped with Saran wrap (no vacuum) and placed in a freezer at −20°C. Samples were taken at 0, 2, 6, 10, and 16 wk of storage, and TBA test was performed.

The last 30 patties of each treatment were precooked at 175°C for 5 min on a griddle and placed on plastic foam trays, wrapped with Saran wrap (no vacuum) and placed in a refrigerator at 4°C. Samples were taken at 0, 2, 5, and 8 days of storage; the TBA test was performed on duplicate samples according to the method of Tarladgis et al. (1960). The TBA number, expressed as mg malonaldehyde per 1000g meat, was determined by multiplying the absorbancy by a factor of 7.8.

Data were analyzed with analysis of variance and Duncan's multiple range test (Steel and Torrie, 1960).

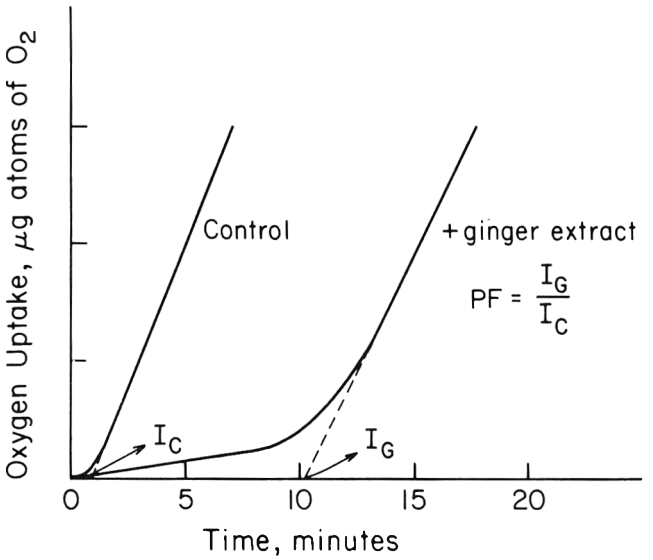

Fig. 1—A typical oxygraph curve. I_C = induction period of control, I_G = induction period of incubation mixture with 0.02 mL ginger extract, PF = protection factor.

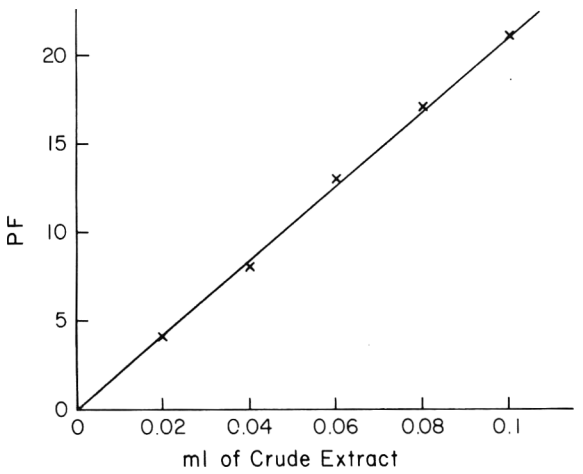

Fig. 2—Relationship between ginger extract (1/10 diluent of crude extract) and protection factor values at pH 6.0.

RESULTS & DISCUSSION

Effect of ginger extract concentration

A typical Oxygraph curve is shown in Fig. 1. In the absence of ginger extract, the addition of metmyoglobin (MetMb) immediately induced the rapid oxidation of the linoleic acid emulsion. The length of induction period was normally 20 sec or less. In contrast, the addition of ginger extract to the system markedly extended the induction period, indicating a strong antioxidant property. The calculated PF values increased linearly with the increase of added ginger extract (Fig. 2).

Distribution of antioxidant property in rhizome

The cross-section of a ginger rhizome shows two distinct regions; the outside peripheral region with greenish color and the inside core region with whitish color. It has been shown that these two regions have markedly different biological activities (Lee et al., 1984). In the comparison of antioxidant effect, however, very little difference was found between these two regions (Table 2), indicating a uniform distribution of antioxidant property throughout the rhizome.

Table 2—Antioxidant property in two different cross-section areas of ginger rhizome

Crude extract[a]	PF Values[b]
Blank	1
0.01 mL outside peripheral area	4.8
0.01 mL inside core area	4.5
0.02 mL outside peripheral area	11.5
0.02 mL inside core area	12.5

[a] Crude extracts from outside peripheral area and inside core area were prepared separately and diluted ten times with distilled water. 0.01 mL or 0.02 mL of these diluted extracts was used in the oxygen uptake study.
[b] PF (protection factor) values represent means of four replications. No significant differences in PF values between two different areas.

Table 3—Antioxidant property in the supernatant and precipitate of crude ginger extract

Preparation added to the system	PF Values[a]
Blank	1
0.02 mL whole homogenate	16.7[b]
0.02 mL supernatant	9.2[c]
0.02 mL reconstituted precipitate	1.5[d]
0.04 mL whole homogenate	40.2[b]
0.04 mL supernatant	19.2[c]
0.04 mL reconstituted precipitate	1.5[d]

[a]PF (protection factor) values represent means of four replications
[b,c,d]Means with different superscripts within the same volume differ significantly ($p<0.01$).

Table 4—Heat stability of antioxidant property in ginger

Duration of heating at 100° C, min	PF Value[a]	Relative potency
0	21.8[b]	100.0
10	17.6[c]	80.7
30	16.0[c,d]	73.3
60	14.5[d]	66.5
120	14.8[d]	67.8

[a] PF (protection factor) values represent means of four replications.
[b,c,d]Means with different superscripts differ significantly ($p<0.05$).

Supernatant vs precipitate of crude extract

The centrifugation of crude extract at 20,000 × *g* produced two fractions: a grayish-green supernatant and a white precipitate. The precipitate alone appeared to have little antioxidant activity, whereas the supernatant retained most activity (Table 3). More interestingly, however, the whole crude extract exhibited almost twice the activity in the supernatant. This suggested that the precipitate contained as yet unidentified component(s) which greatly enhanced the antioxidant activity present in the supernatant.

Heat stability

The crude extract was heated in a boiling water bath for 0, 10, 30, 60 and 120 min, and the residual antioxidant activity was determined. Heating at 100°C for 10 min significantly ($P<0.05$) reduced antioxidant potency by 20% (Table 4). Heating for 30 min or longer further reduced the activity but at a much slower rate, and little change was observed between 60 and 120 min. The results indicated that antioxidant in ginger rhizome was fairly heat stable with 2/3 activity still remaining after prolonged heating at 100°C.

Effect of pH

The antioxidant effectiveness of ginger extract gradually increased with maximum values at pH 7.0 at low concentrations (0.02 and 0.03 mL) of extract (Fig. 3). At alkaline pH, the PF values either decreased or continuously increased depending on the amount of ginger extract added to the system.

It has been well documented that hemoprotein-catalyzed oxidation is most active at alkaline pH (Liu, 1970a). Lee et al. (1975) also reported that oxygen uptake rate did not change between pH 5.0 and 7.0, but increased two and four times at

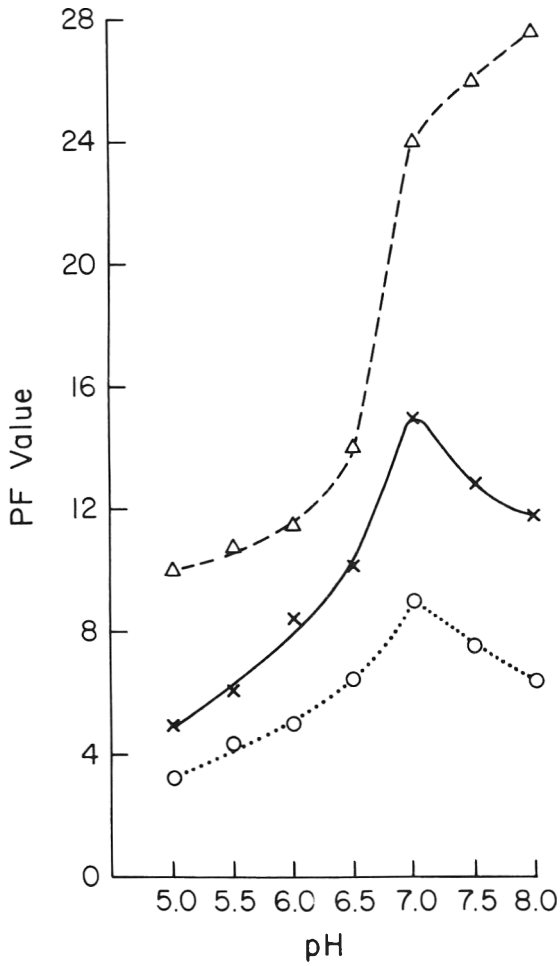

Fig. 3—Effect of pH on antioxidative effectiveness of ginger extract: ○· · ·○ 0.02 mL extract; ○—○ 0.03 mL extract; △—△ 0.04 mL extract.

pH 7.5 and 8.0, respectively. Hence the decrease of PF values at alkaline pHs at 0.02 and 0.03 mL extract was not due to the loss of antioxidant property, but could be due to the marked enhancement of lipid oxidation. The low concentration of ginger extract may not have been high to counteract the acceleration of heme catalyzed oxidation, resulting in decrease of PF values at alkaline pH.

Changes of TBA values during storage of pork patties

For the refrigerated fresh pork patties, there was a linear increase of TBA number during storage regardless of treatments (Fig. 4). However, a marked difference was observed in the rate of increase among treatments, T_1 (0% extract) showing a 2.5 times greater rate than T_5 (0.5% extract). The result demonstrates that ginger extract was effective in retarding the development of rancidity in salted pork patties and its effectiveness was directly related to the concentration. The relatively rapid increase of TBA in the control, compared to previous reports, would be attributed to the presence of salt, which is a prooxidant (Love, 1983; Govindarajan et al., 1977).

The change of TBA value during storage of the precooked refrigerated pork patties is illustrated in Fig. 5. The rate of increase in TBA number was approximately 2.5 times greater than in uncooked patties. The accelerating effect of heating on the development of oxidative rancidity in meat and meat products has been well documented by many researchers (Younathan and Watts, 1959; Sato and Hegarty, 1971).

As in the case of fresh pork patties, ginger extract exhibited a strong antioxidant property in precooked patties. It took T_5 (0.5% extract) three times longer period to reach the same TBA

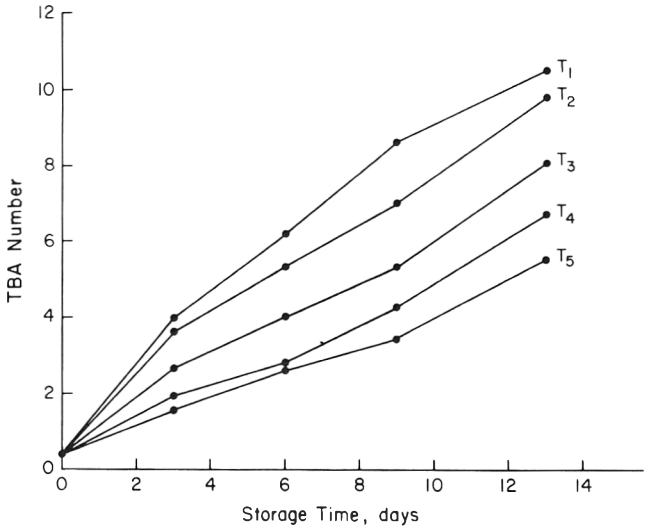

Fig. 4—Changes in TBA value during refrigerated storage (4°C) of fresh pork patties. $T_1 = 0\%$, $T_2 = 0.05\%$, $T_3 = 0.1\%$, $T_4 = 0.25\%$, $T_5 = 0.5\%$ ginger extract.

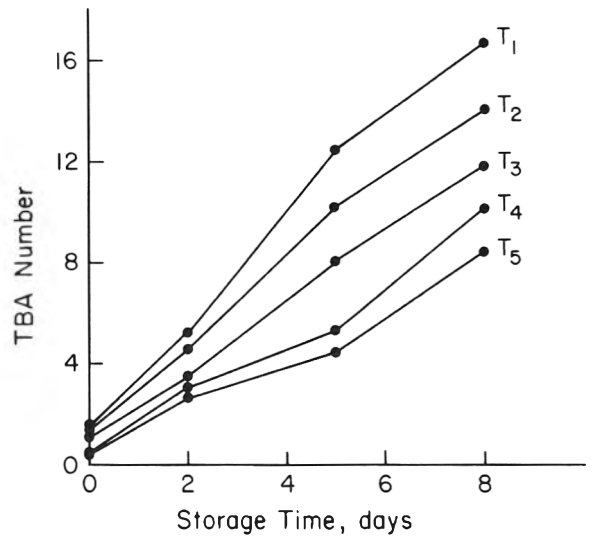

Fig. 5—Changes in TBA value during refrigerated storage (4°C) of precooked pork patties. $T_1 = 0\%$, $T_2 = 0.05\%$, $T_3 = 0.1\%$, $T_4 = 0.25\%$, $T_5 = 0.5\%$ ginger extract.

number as that of T_1 (no extract). The retardation of lipid oxidation by adding ginger extract would be beneficial for the fast food facilities serving precooked meat. In oriental countries ginger has been extensively used as an essential spice in the cooking of beef and pork. Average consumers in those countries appreciate the benefits of using ginger as a flavoring agent, but the traditional heavy usage may also have been effective in the extension of shelf life, especially in the absence of refrigerators.

The change of TBA value during frozen storage of salted pork patties is shown in Fig. 6. The antioxidant property of ginger extract was again manifested in frozen pork patties. The retardation of TBA value increase was proportional to the amount of ginger extract added to the product. As the storage time increased, however, the difference in TBA number between the control and the treated samples became less. This was due to the fact that the TBA value in the control did not increase linearly but leveled off after reaching 8 mg malonaldehyde per kg meat, whereas it increased continuously in the treated products. This trend was quite different from that of refrigerated fresh or precooked patties which showed a linear increase of TBA number beyond the value of 8. Other investigators re-

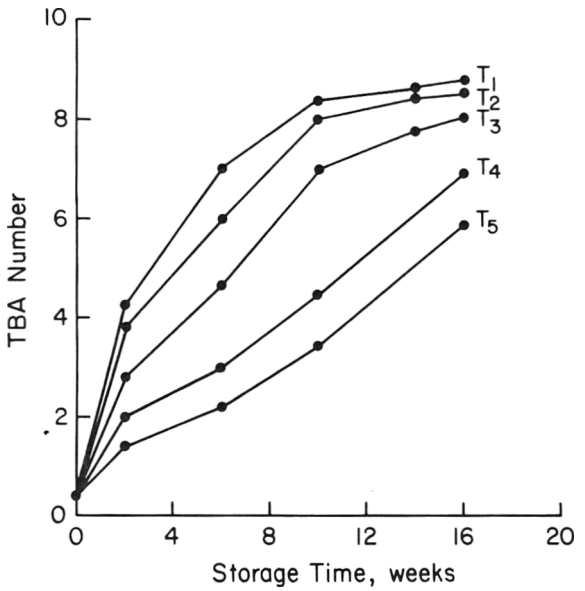

Fig. 6—Changes in TBA value during frozen storage (−20°C) of pork patties. $T_1 = 0\%$, $T_2 = 0.05\%$, $T_3 = 0.1\%$, $T_4 = 0.25\%$, $T_5 = 0.5\%$ ginger extract.

ported the decrease of TBA value after an extended storage of raw meat and subsequent cooking (Benedict et al., 1975; Igene et al., 1979).

In conclusion, the present study demonstrated that ginger rhizome contained a potent antioxidant activity against heme-catalyzed lipid oxidation. The antioxidant effectiveness was dependent on the kinds of preparation, pH, and concentrations. When tested with pork patties, the storage stability of all products determined by TBA value was improved by the inclusion of ginger extract.

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uting to the functionality of the products by reducing weight losses in formulations containing the lower level of NaCl.

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Color, Doneness and Soluble Protein Characteristics of Dry Roasted Beef Semitendinosus

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ABSTRACT

Subjective color and doneness profiles, objective color measurements and compositional changes in water and low-salt extractable protein fractions of beef semitendinosus roasts cooked to 60°C, 68°C, and 75°C internal temperature (IT) were evaluated. Sensory color scores showed higher significant correlations with IT than objective color measurements. Extractable biuret positive ratio (EBPR) and coagulation test values had the highest significant correlations with IT compared to related water and saline-extractable protein parameters. Intensity profiles of isoelectrofocused water-extractable protein aliquots indicated that proteins with pI 3–10 decreased as IT increased. Proteins with pI 6.2–8.0 also accounted for an increase in percent of the total protein extracted as IT increased.

INTRODUCTION

COOKING of dry-roasted beef requires a fine balance between adequate heating to hydrolyze collagen, minimum heating to obtain rare color quality and to minimize myofibrillar toughening, and maximum heating to ensure thermal inactivation of microorganisms. For commercial processors of meat products for restaurant/institutional markets, this balance means maintaining color quality that consumers associate with preferences for degree of doneness (rare, medium, well done). For regulatory agencies, it means adequate laboratory testing methodology to monitor heat processing, thereby ensuring consumer safety and health. Time and temperature, color and state of doneness and protein reactions to heat are all important factors in basic studies of the reactions of muscle constituents during heating and effects on cooked meat quality.

Color is generally used as evidence of extent of heat exposure in cooked beef. Color and doneness in relation to temperature have often been subjectively assessed by individual researchers (Cover et al., 1957; Bernofsky et al., 1959; and Visser et al., 1960); for objective color, measurements have been related to the visual redness associated with doneness (Ferger et al., 1972; Shaffer et al., 1973; Fogg and Harrison et al, 1975; Blankenship et al., 1980).

Other studies on heat exposure of cooked meat have focused on general solubility loss of proteins as they denature/coagulate (Hamm and Deatherage, 1960; Cohen, 1966; Crespo and Ockerman, 1977; Davis and Anderson, 1983, 1984). Food Safety and Inspection Service (FSIS, 1981) monitors compliance with temperature requirements for cooking beef roasts by a coagulation test (Coretti, 1957) which is based on protein solubility loss. Electrophoresis (Lee et al., 1974; Steele and Lambe, 1982; Caldironi and Bazan, 1980), differential scanning calorimetry (DSC) (Martens et al., 1982), and size exclusion/HPLC (Davis and Anderson, 1984) have been used to further study the reactions of muscle protein constituents to heat exposure.

The objective of this study was to examine the relationships among subjective color and doneness profiles, objective color measurements, and compositional changes in water and low-salt (0.9%) extractable protein fractions (which include proteins responsible for color) of beef semitendinosus muscle heated to three internal temperatures using two oven heating rates.

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MATERIALS & METHODS

Cooking procedures

Roasts (m. semitendinosus), weighing 2.2–3.2 kg, were purchased from local supermarkets, trimmed of excess fat and connective tissue, and sub-divided into three individual roasts (500–700g each). Two sets of three roasts were assigned to target internal temperatures of 60°C, 68°C, and 75°C, and cooked in ovens set at 121°C (250°F) and 177°C (350°F). Cooking was replicated four times, alternating the oven temperatures between the two conventional ovens used in the study. Internal temperatures were measured by copper-constantan thermocouples placed in the center of the roasts and recorded on a Honeywell recording 24-point potentiometer (Honeywell, Fort Washington, PA).

Evaluation of color and doneness

At each of the four cook sessions, 15 panelists (age range 23–55) evaluated visual color and degree of doneness of roast center slices (edges trimmed). Use of the score sheet and the procedural mechanics of visually observing the color of the meat slice, matching the perceived color with color descriptors provided on the scoresheet, and marking the doneness scale to describe the degree of doneness of each meat slice were explained during orientation and training. Color terms provided on the scoresheet were very red, medium red, pink, slightly pink, pinkish gray, gray brown and brown. Doneness terms were rare, medium rare, medium, medium well and well-done.

Two center slices (5 mm thick) of each cooked roast were made. One slice from each roast per replication was allowed to "bloom" for 10 min and then immediately placed in a specially designed booth with six compartments providing 100 ft-c of lighting. The order of placement of the six roast slices was randomized at each viewing session. Operation of panel testing was carefully managed to have all panelists arrive at the same time and to one-by-one view the slices and mark responses on their scoresheets.

Objective color measurements on the second center slice included K/S values (Judd and Wyszecki, 1963) calculated from absorbance readings at 525 nm and 572 nm on a Bausch and Lomb Spectrophotometer, Model 505, with reflectance attachment (Bausch and Lomb, Rochester, NY) (Stewart et al., 1965). Hunter L, +a and +b values (Hunter Color and Color Difference Meter, Model D-25-D, Fairfax, VA) were recorded. From these values, hue angle ($\tan^{-1}$ b/a) (Little 1975) and saturation $(a^2 + b^2)^{1/2}$ were calculated.

Protein extracts

Surfaces of roasts were trimmed about 0.65 cm to remove external fat and surface browning. Ground meat (3 times, 0.45 cm plate) was blended with 0.9% saline solution (ratio 1:2, w/v). After 30 min, blended samples were filtered first through #541 Whatman filter paper with a pad of Celite filtering aid and again through Whatman #42 filter paper and celite cover pad under vacuum. Protein content (mg protein/mL extract) of the filtered extract was determined using the Biuret procedure (Layne, 1957). An aliquot of the initial extract was reheated to 70°C for 15 min, filtered and another biuret protein value

determined. The ratio of biuret values of the initial extract and reheated (to 70°C for 15 min) extract was expressed as extractable biuret positive ratio (EBPR) (Davis and Anderson, 1983).

Aliquots of the saline extract were used to conduct the coagulation test (FSIS, 1981; Coretti, 1957). Percentage transmittance (670 nm) of the extracts was measured before and after heating on a Spectronic 710 spectrophotmeter (Bausch and Lomb, Rochester, NY) to observe turbidity changes.

A water extract of ground roast was made (1:2 ratio, sample to deionized H_2O, w/v) and filtered as described by Davis and Anderson (1983). Aliquots of these water-extractable protein fractions, equal to 0.01 mg protein based on Biuret protein measurements, were applied to thin-layer (0.8 mm) polyacrylamide gels, pI range 3–10, and isoelectrofocused at constant power of 15 watts, 1300 volt limit. Protein standards (Bio-Rad Laboratories, Richmond, CA) in the pI range 3–10 were also applied as markers. Separated proteins were fixed and stained with Bio-Rad Silver Staining Kit. Preserved gels were scanned on a laser densitometer (LKB, Houston, TX) to obtain intensity profiles and protein migration distances.

Data Analyses

ANOVA procedures (SAS, 1982), were used to test for variances in data due to internal temperature (IT), oven temperature (OT) and IT × OT interaction. Data for each parameter were regressed on maximum post-oven temperature attained and heating rate (min/g) by General Linear Model (GLM) (SAS, 1982) to observe linear and/or quadratic relationships.

ANOVA data indicated that all parameters were significant for internal temperature (IT) (Table 1) but not for oven temperature (OT) or IT × OT interaction. Quadratic models were nonsignificant ($P>0.05$); and where linear relationships were significant, slopes for data from 121°C oven vs 177°C oven were tested for differences but none was observed. Therefore, values for measured parameters were pooled over oven temperatures and simple correlation coefficients among all parameters were calculated.

RESULTS

Sensory and objective color and doneness

FIGURE 1 reports the panelists' definitions of doneness by color descriptions. Percentages were derived from the number of times a color term was paired with a doneness term when evaluating sample slices divided by the total frequency for the doneness term in the study. Definitions of medium rare, medium and medium well were not as clearly discriminated by one or two color terms as were rare and well-done. Frequency distributions of color and doneness terms by IT revealed clustering of terms at either end of the scales for 60°C and 75°C roasts, while responses for 68°C were distributed among midscale terms (Table 2). Sensory color and doneness descriptions were converted to scores to analyze for differences due to IT. The proportions of variation accounted for in the linear model due to IT were $R^2 = +0.90$ for sensory color evaluations and $R^2 = +0.92$ for doneness evaluations (Table 1). As IT increased, color and doneness scores increased (i.e., less red and more done).

There were significant differences ($P<0.05$) in objective color values among roasts at the three internal temperatures for each of the color parameters measured (Table 1). Hunter L values (lightness) increased with increasing IT. Both redness (+a values) and yellowness (+b values) decreased with increase in IT. Hue angle increased (less redness) and saturation (color strength) decreased with increase in IT. Decrease in ratios of K/S value at 572 nm to K/S value at 525 nm with increase in IT indicated higher content of brown vs red pigment (Stewart et al., 1965).

Soluble protein changes

Soluble protein content of the extracts (0.9% salt), as mg protein/mL extract, decreased with increase in IT. Biuret extractable positive compounds were 4.67, 3.28, and 2.85 mg protein/mL extract, respectively, for internal temperatures of 60°C, 68°C, and 75°C. EBPR values of heated and reheated (70°c, 15 min) extracts averaged 1.51, 1.15, and 1.09, respectively, for roasts cooked to 60°C, 68°C, and 75°C (Table 1).

Generally, extracts obtained from roasts cooked to 60°C were red and uncloudy; 68°C roast extracts were less red; and 75°C roast extracts were virtually clear and colorless. The initial percent transmittance increased as IT increased ($P<0.05$), indicating fewer soluble solids in the extracts from roasts at higher IT. Changes in transmittance due to cloudiness caused by aggregation and coagulation of proteins as the extracts were heated in a water bath for the coagulation test were similar for the three roast temperatures, averaging 20–24 units of change. The ratios of % transmittance before heating to % transmittance after heating (1.36, 1.28, and 1.31, respectively for 60°C, 68°C, and 75°C roasts) followed a similar trend as the EBPR values. Both the EBPR and transmittance ratios relate to the same phenomena (coagulation/aggregation of remaining soluble protein on reheating) and would indicate that subjective notations of the coagulation test were fairly reliable in this study. Average temperatures recorded for the coagulation temperature (i.e., onset of cloudiness) were slightly lower than the maximum IT recorded (Table 1).

IEF protein patterns

Figure 2 represents the intensity profiles obtained from densitometry scanning of isoelectrofocused gels of extracts from roasts at 60°C, 68°C, and 75°C cooked at 121°C. Changes in the proportions of the individual proteins making up the total

Table 1—Statistical values of analysis of variance, mean separation, and simple correlation of dependent variables for tests of differences due to internal temperature

	Analysis of variance			Mean Values*			
	Internal temperature		Proportion of variation accounted for in data	Target Temperature °C			Correlation coefficients
Dependent variable	F-value	Probability	R^2	60	68	75	(vs Internal temp)
Sensory Color[y]	63.26	<0.01	0.90	1.85[a]	3.85[b]	5.75[c]	+0.90
Sensory Doneness[y]	77.11	<0.01	0.92	1.55[a]	2.85[b]	4.20[c]	+0.91
Hunter L	7.60	<0.01	0.59	48.56[a]	52.50[b]	52.82[b]	+0.56
Hunter +a	42.25	<0.01	0.87	16.24[a]	11.46[b]	6.16[c]	−0.85
Hunter +b	12.99	<0.01	0.69	13.29[a]	13.22[a]	11.05[b]	−0.65
Hue angle	42.24	<0.01	0.87	39.58[a]	50.05[b]	61.50[c]	+0.84
Saturation	16.56	<0.01	0.67	20.03[a]	17.60[ab]	12.59[b]	−0.64
K/S ratio	27.84	<0.01	0.83	1.31[a]	1.22[b]	1.01[c]	−0.76
mg protein/ml extract	17.64	<0.01	0.80	4.67[a]	3.28[b]	2.85[c]	−0.68
Biuret ratio (EBPR)	30.64	<0.01	0.82	1.51[a]	1.15[b]	1.09[b]	+0.80
Coagulation temp. (° C)	34.51	<0.01	0.85	59.50[a]	67.30[b]	72.25[c]	+0.86
Initial %T - Extract	8.21	<0.01	0.71	90.35[a]	93.35[ab]	96.15[b]	+0.58
%T Extract after heating	3.59	0.05	0.74	66.30[a]	73.00[b]	73.75[b]	+0.35

[a,b,c] Values on a line with different superscripts are significantly different ($P<0.05$).

* Values are the means of 8 or more observations for all dependent variables except sensory color and doneness which had 112 observations.

[y] Color scale: 1=very red to 7=brown; Doneness scale: 1=rare to 5=well done.

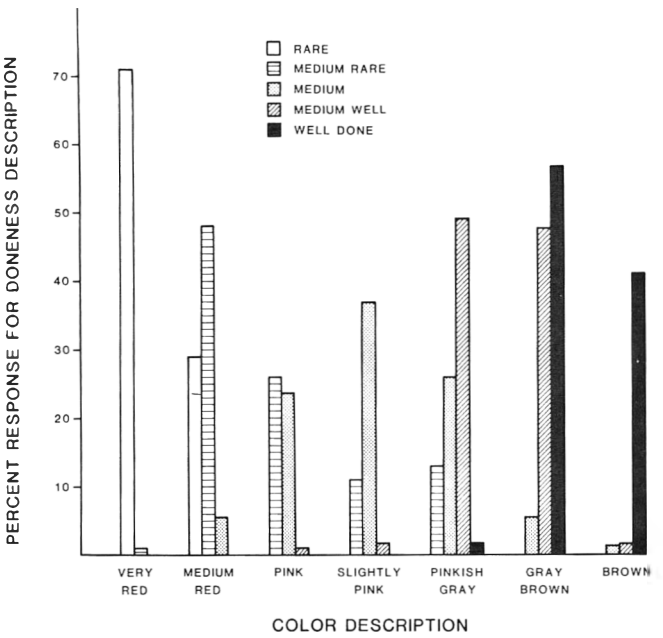

Fig. 1 — Panelists pairing of doneness terms with color description terms (by percent of total responses for each doneness term).

Table 2—Frequency distributions of color and doneness descriptions by temperature

Sensory scale	Temperature °C 60	68	75	Row total
Color Scale				
1 Very red	45	0	0	45
2 Medium red	50	13	2	65
3 Slightly pink	9	26	6	41
4 Pink	5	29	4	38
5 Pink-Gray	3	38	20	61
6 Gray-Brown	0	0	56	62
7 Brown	0	0	24	24
Column total	112	112	112	336
Doneness				
1 Rare	59	3	0	62
2 Medium rare	46	35	8	89
3 Medium	7	51	15	73
4 Medium-well	0	23	36	59
5 Well-done	0	0	53	53
Column total	112	112	112	336

[a] Row and column totals = 336, instead of 360 (6 roasts × 4 replications × 15 panelists) due to elimination of data of one panelist with a missing replication.

protein quantity extracted were evident in the scans. In the scanning range 30–50 mm from the cathode, fewer bands disappeared and the percentage of the remaining peak areas to the whole increased from 36% at 60°C, to 58% at 68°C and to 78% at 75°C. By extrapolation and visual comparisons to pI markers, the 30–50 mm portion of the intensity profiles coincided with pI range 6.2–8.0. The isoelectric points of horse and whale myoglobin bands in the pI marker sample were 8.2 and 7.0, respectively.

Simple correlations among parameters

Almost all parameters exhibited significant ($P<0.05$) relationships to other parameters and, in general, those with the highest correlations vs internal temperature ($r \geq 0.90$) were objective and sensory color and doneness parameters (Table 1). Visual assessment of color and perception of doneness had a correlation of $r = +0.99$, indicating that these two parameters could be interchangeable. There were also high correlations between sensory color or doneness and Hunter +a values (redness) ($r = -0.91$) and hue angle ($r = +0.92$). Hunter +a values were highly correlated with K/S ratio ($r = +0.92$). However, the correlation between K/S ratio and sensory color was $r = -0.77$ and between K/S ratio and doneness was $r = -0.79$.

Protein concentration of the extract (mg protein/mL) was highly correlated with initial % transmittance ($r = -0.93$) and with coagulation temperature ($r = -0.82$). Coagulation temperature was highly correlated with maximum temperatures ($r = +0.86$), with sensory color ($r = +0.90$) and sensory doneness ($r = +0.89$).

DISCUSSION

TWO GROUPINGS of parameters emerged as distinguishing differences among roasts at all three temperatures. One group included those parameters that related to color by visual sensory assessment or objective surface measurements of slices from cooked meat (sensory color and doneness, Hunter +a values, hue angle, K/S ratio). A second group of parameters that distinguished among all three temperatures included Biuret ratio and coagulation test values. These related to protein components (sarcoplasmic, water-soluble fractions) that are primary reactors in color change in meat.

The absence or reduction in red color of slices of cooked roasts as temperature increased was evident from panel observations and objective color measurements. The panel scores were more highly correlated with IT than were objective values for meat color determinations. However, descriptions of color and doneness at 68° C were less well-defined than descriptions for 60°C and 75°C roasts. The objective measurements that discriminated among IT's (i.e., +a values, hue angle, K/S values) were all related to decreasing redness. Some workers have explained the absence of red color in cooked meat as the denaturation of myoglobin and the formation of hematin compounds (Ledward, 1971). Davis and Anderson (1984) showed that myoglobin evaluated by size exclusion/HPLC in constant sample volumes of water extracts from cooked ground beef decreased with time and temperature and essentially was absent at 75°C. Martens et al. (1982) suggested that native red myoglobin denatures in the temperature range 65–80°C with 70% remaining at 73°C (based on DSC data of purified myoglobin

Table 3—Correlation coefficients of selected dependent variables.[x]

	Hunter L	Hunter +a	Hue angle	K/S ratio	Sensory color	Sensory doneness	Coagulation temp	Initial % T	Biuret value (mg protein/mL)
Hunter +a	−0.51								
Hue angle	+0.52	−0.98							
K/S Ratio	−0.46	+0.93	−0.91						
Sensory color	+0.60	−0.90	+0.84	−0.77					
Sensory doneness	+0.60	−0.91	+0.83	−0.79	+0.99				
Coagulation temp	+0.46	−0.85	+0.89	−0.71	+0.90	+0.89			
Initial % T	+0.43	−0.70	+0.70	−0.63	+0.69	+0.68	+0.71		
Biuret value (mg protein/mL)	−0.53	+0.70	−0.72	+0.59	−0.77	−0.75	−0.82	−0.93	
EBPR (Biuret ratio)	−0.62	+0.71	−0.71	+0.60	−0.77	−0.76	−0.75	−0.78	+0.87

[x] Correlation coefficients of 0.40 or higher were significant at $P < +0.05$.

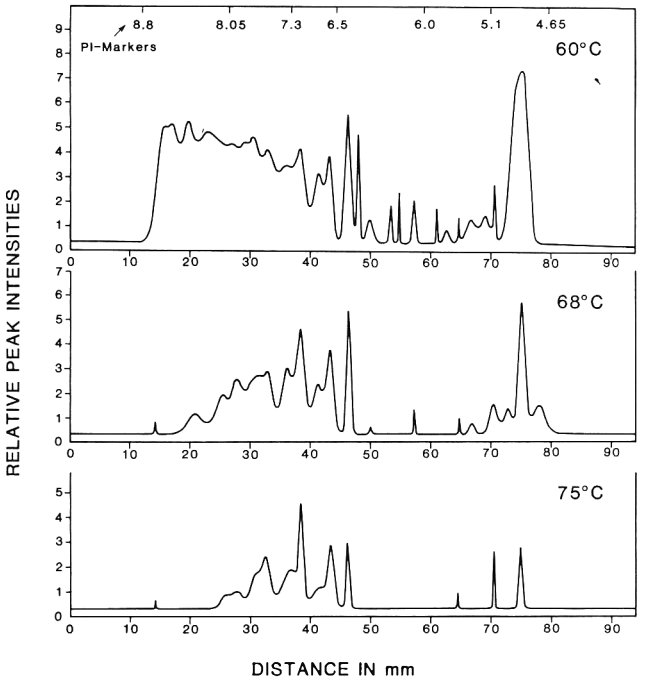

Fig. 2 — Intensity profiles (laser densitometer, LKB) of IEF gels from water extracts of roasted (oven at 121°C) beef semitendinosus at 60°C, 68°C, and 75°C.

solution). These authors further suggested that red color in cooked meat appears to diminish at lower temperatures due to a masking effect of myoglobin by aggregation/co-precipitation of other sarcoplasmic and myofibrillar proteins.

The reduction in extractable protein (Biuret values) as IT increased is evidence of solubility loss due to heating. Further evidence of solubility loss and an aggregation phase in the course of heat denaturation is shown in data from the coagulation test, by changes in turbidity (% transmittance), and EBPR values. The aggregation could be a source of masking of red pigment and/or co-precipitation of myoglobin with other proteins, thereby changing surface light scatter characteristics that affect visual and objective assessments of color in cooked meat, especially at 68°C IT. Hunter L values (lightness) indicated significant ($P<0.05$) lightness differences between 60°C and 68°C, but not between 68°C and 75°C (remained about the same).

The densitometric scans of IEF gels in this study (Fig. 2) showed that some water-extractable protein remained at 75°C. The largest peak areas of the 75°C extract scans represented more than 70% of the soluble protein remaining in this sample. This probably represents the baseline of extractable protein shown by Davis and Anderson (1983). Based on pI marker standards, this area of the scan represented protein fractions that might include some form of the color pigment myoglobin or other hemochromes with similar pI (i.e., pI 7.2–8.0).

Subjective color/doneness evaluations were more highly correlated with IT than were objective color measurements of roast beef slices. Objective analytical procedures of protein fractions extracted from roast beef slices indicate that more work is needed to understand the basic muscle protein constituent reactions to heat, including denaturation rates and temperatures of purified and native tissue myoglobin and its derivatives. The effect of different heat denaturation rates of the protein components in muscle (including the colorless sarcoplasmic and myofibrillar proteins) on perceived color and doneness needs to be explored. Further studies should also emphasize comparisons of model systems versus intact muscle.

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Effect of Low-Calcium-Requiring Calcium Activated Factor on Myofibrils under Varying pH and Temperature Conditions

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ABSTRACT

The effect of low-calcium-requiring calcium-activated factor (μM CAF) on the myofibrils under varying pH at 5°C and 25°C was examined spectrophotometrically (absorbance at 278 nm), electrophoretically (sodium dodecyl sulfate polyacrylamide gel electrophoresis), and microscopically (phase microscopy and transmission electron microscopy). Results indicated that at conditions similar to those of postmortem storage (i.e., pH 5.5–5.8 and 5°C), μM CAF retained 24–28% of its maximum activity (pH 7.5 at 25°C). This 24–28% of maximum activity was sufficient to reproduce most of the known changes associated with the tenderization process during postmortem aging. It was concluded that because of the activity of μM CAF under postmortem conditions, it seems reasonable to suggest that μM CAF may be responsible, in part, for some of the postmortem changes observed.

INTRODUCTION

AGING OF CARCASSES after slaughter for 8–14 days at 0–4°C, to improve their tenderness, has been practiced for many years and still remains an important procedure for producing tender meat. Although the increase in meat tenderness is measurable both subjectively and objectively, the mechanism(s) of this tenderization process still remains an unresolved issue for meat scientists.

Tables 1 and 2 summarize the changes observed in myofibrils as a result of postmortem storage and the effects of calcium-activated factor (CAF) on myofibrils, respectively. Comparison of these two tables clearly indicates that CAF is capable of producing many changes that are associated with aged muscle. In spite of a remarkable resemblance between the effect of postmortem aging and CAF treatment on myofibrils, several legitimate questions have been raised suggesting that CAF may not be responsible for the changes observed as a result of postmortem storage. First, high-Ca^{++} requiring form of CAF (mM CAF) is maximally active at pH 7.0–8.0 (Dayton et al., 1976b) and, therefore, has very little activity at pH 5.5–5.8 (postmortem condition). The question arising at this point is, whether CAF must be maximally active to cause sufficient damage to the myofibrils to produce the observed changes during postmortem again. Marsh (1981) reported that if only one sarcomere in every 250 was broken, such a change (even though undetectable) could result in a significant improvement in tenderness. Therefore, CAF does not need to be maximally active to bring about postmortem changes in muscle. After all, if CAF was maximally active almost all of the Z-disks would be degraded (Koohmaraie, 1984a), a change that is never associated with postmortem aging.

Table 1—Changes associated with postmortem tenderization at 2–4°C

1. Z-disk weakening (Henderson et al., 1970; Olson et al., 1976) and myofibril fragmentation.
2. Disappearance of troponin-T and appearance of a 30,000-dalton component (MacBride and Parrish, 1977; Olson et al., 1977; Koohmaraie et al., 1984a, b, c).
3. Disappearance of desmin (Young et al., 1981; Koohmaraie et al., 1984a, b, c).
4. Appearance of a 95,000-dalton component (Koohmaraie et al., 1984a, b, c).
5. Disappearance of titin (Takahashi and Saito, 1979).
6. The major contractile proteins, myosin and actin, are not affected (Olson et al., 1977; Penny, 1980; Koohmaraie et al., 1984a, b, c,).

Table 2—Effects of CAF on myofibrils

1. Z-disk degradation (Dayton et al., 1975, 1976a, b; Koohmaraie et al., 1984a).
2. Disappearance of troponin-T and appearance of a 30,000-dalton component (Dayton et al., 1975; Olson et al., 1977; Koohmaraie et al., 1984a).
3. Appearance of a 95,000-dalton component (Koohmaraie et al., 1984a).
4. Loss of desmin (O'Shea et al., 1979; Zeece et al., 1983; Koohmaraie et al., 1984a).
5. Titin is quickly degraded (Penny et al., 1984).
6. The major contractile proteins, myosin and actin, are not affected (Dayton et al., 1975; Olson et al., 1977).

Secondly, mM CAF is maximally active at 1–5 mM concentration of free Ca^{++} (Dayton et al., 1976b). The reason for concern in this instance is that free intracellular Ca^{++} concentrations would never reach this level and therefore CAF cannot be activated during postmortem storage. This argument was correct until the discovery of the second form of CAF, low-Ca^{++} requiring CAF (μM CAF). Although intracellular free Ca^{++} concentration is thought to be only 1–10 μM, the Ca^{++} concentration increases gradually with increasing time of postmortem storage and may reach levels at which μM CAF can be activated (Goll et al., 1983).

On the basis of these arguments as well as others (Goll et al., 1983), it would seem unlikely that mM CAF is responsible for postmortem changes. If CAF is involved, it must be the low-Ca^{++} requiring form of CAF, due to its low Ca^{++} requirements and also its broader range of pH activity (Dayton et al., 1981). In view of the lack of any experimental data regarding roles of μM CAF in postmortem aging, this study was conducted to examine the effects of μM CAF on myofibrils at conditions that exist during postmortem aging of meat (i.e., pH 5.5–5.8 and 5°C).

MATERIALS & METHODS

Extraction and purification of low-calcium-requiring CAF

Low-calcium-requiring CAF was prepared according to the procedure described by Dayton et al. (1981). Briefly, chilled muscle (4,000g) was ground, suspended in 2.5 volumes of 50 mM Tris-acetate, pH 8.0, 4 mM ethylene-diaminetetraacetate (EDTA), homogenized and centrifuged at 15,000 × g_{max} for 30 min. The resulting supernatant was salted out between 30–40% saturation with ammonium sulfate. The precipitate was dissolved in 20 mM Tris-acetate, pH 7.5, 1 mM

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EDTA, 10 mM 2-mercaptoethanol (MCE) and dialyzed against the same buffer for 12 hr. This solution was clarified at 105,000 × g_{max} for 60 min and chromatographed on DEAE-Sephacel that had been equilibrated against dialysis buffer. The bound protein was eluted with a continuous gradient of 10–400 mM KCl in dialysis buffer. Fractions with calcium-activated proteolytic activity at 100 μM Ca^{++} (Peak I of Dayton et al., 1981) were pooled and salted-out between 0–45% saturation with ammonium sulfate. The precipitate was dissolved in 20 mM Tris-acetate, pH 7.5, 1 mM EDTA, 1 mM NaN_3 and chromatographed on Ultrogel AcA-34 (LKB Instruments, Inc.). Fractions containing calcium-activated proteolytic activity at 100 μM Ca^{++} were pooled and then subjected to a second DEAE-Sephacel column. The bound protein was eluted with a continuous gradient from 0–200 mM KCl in dialysis buffer.

Protein concentration

Protein concentrations were determined by the biuret method (Gornall et al., 1949) or by the protein-dye binding method (Bradford, 1976).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was conducted according to the procedure described by Porzio and Pearson (1977) using 10% gels. Myosin, β-galactosidase, phosphorylase B, bovine albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme were used as protein standards.

Myofibril isolation

Myofibrils were prepared immediately after slaughter from sternomandibularis muscle according to the procedure described by Goll et al. (1974) using 100 mM KCl, 50 mM Tris-HCl, pH 7.6, and 5 mM EDTA as the isolating medium.

Assay for μM CAF activity

Calcium-activated proteolytic activity was assayed using either casein (Hammerstein) or purified myofibrils as the substrate at 25°C (or stated temperature) in 100 mM KCl, 50 mM Tris-acetate, pH 7.5 (or stated pH), 10 mM MCE, 100 μM $CaCl_2$ and 5 mg/mL casein or myofibrils. Total reaction volume was 2 mL. Control for enzyme as substrate accompanied each assay. The reaction was initiated by addition of μM CAF, and stopped by addition of 2 mL of 5% trichloroacetic acid (TCA) when casein was used, or 0.22 mL of 100 mM EDTA when myofibrils were used. The assay tubes were then centrifuged at 1,000 × g_{max} for 30 min (when myofibrils were used, centrifugation was 14,000 × g_{max} for 30 min), and the absorbance of the supernatant was measured at 278 nm. To assess the effects of pH and temperature on the substrates alone, each treatment (pH × temperature) was accompanied by its own control. This control tube was used as a blank for the spectrophotometric readings to measure the activity of the corresponding treatment.

Incubation of myofibrils with purified μM CAF

Myofibrils isolated from at-death sternomandibularis muscle were incubated with chromatographically purified μM CAF at the ratio of 1:200 (μM CAF:myofibrils) for 90 min according to the procedure described for measuring μM CAF activity. The reaction was initiated by addition of the μM CAF fraction and stopped by addition of 0.22 mL of 100 mM EDTA. Three sets of tubes were prepared for analysis of all treatments by SDS-PAGE, phase microscopy and electron microscopy. For analysis of the results by SDS-PAGE, myofibrils were sedimented at 2,000 × g_{max} at 4°C and washed three times at 4°C by suspension in 2 mL of 100 mM NaCl and sedimented at 2,000 × g_{max}. The sedimented myofibrils were then dissolved in 2 mL of 1% SDS and boiled in a water bath for 15 min (Dayton et al., 1975).

Electron microscopy

Upon completion of CAF incubation of myofibrils (90 min), the reaction was stopped by the addition of 0.22 mL of 100 mM EDTA. Myofibrils were then pelleted at 3,000 × g_{max} for 10 min. The supernatant was discarded and cold 2% glutaraldehyde, 0.1M cacodylate, pH 7.2, was layered gently over the pellet and allowed to stand undisturbed for 10 min. This step and all subsequent steps prior to addition of the embedding resins were carried out on ice. After 10 min the pellet was gently dislodged, fresh glutaraldehyde was added and samples were cubed (2 mm) with a razor blade. After 1 hr, samples were rinsed three times in 0.1M cacodylate buffer, pH 7.2, and post-fixed in 1% osmium tetroxide, 0.1M cacodylate buffer, pH 7.2 for 1 hr. Sample tubes were then dehydrated in a graded series of acetone and were embedded in an Epon-Araldite mixture and cured overnight at 56°C. Silver-gold sections were cut, using a diamond knife on a LKB Ultratome-5, stained with uranyl acetate and Reynold's lead citrate and examined with a Zeiss EM-10A transmission electron microscope.

RESULTS & DISCUSSION

FIGURE 1 SHOWS the effect of pH on μm CAF activity at 5 and 25°C with casein or myofibrils used as substrate for the protease. Based on Fig. 1, it can be concluded that: (1) μM CAF is more active when myofibrils are used as substrate and (2) μM CAF is, indeed, active at pH 5.5–5.8 and 5°C (24–28% of pH 7.5 and 25°C). The difference in the activity of μM CAF with casein and myofibrils as substrate could partially be explained by a decrease in casein solubility at the lower pH and 5°C. This loss of solubility could, therefore, affect availability of casein as the substrate for the protease (isoelectric point of casein 4.6).

Penny (1980), based on the findings of Dayton et al. (1976b) and Reddy et al. (1975) with high-Ca^{++}-requiring form of CAF, suggested that CAF retained 15–25% of maximum activity between pH 5.5–5.9. Results of this experiment are in

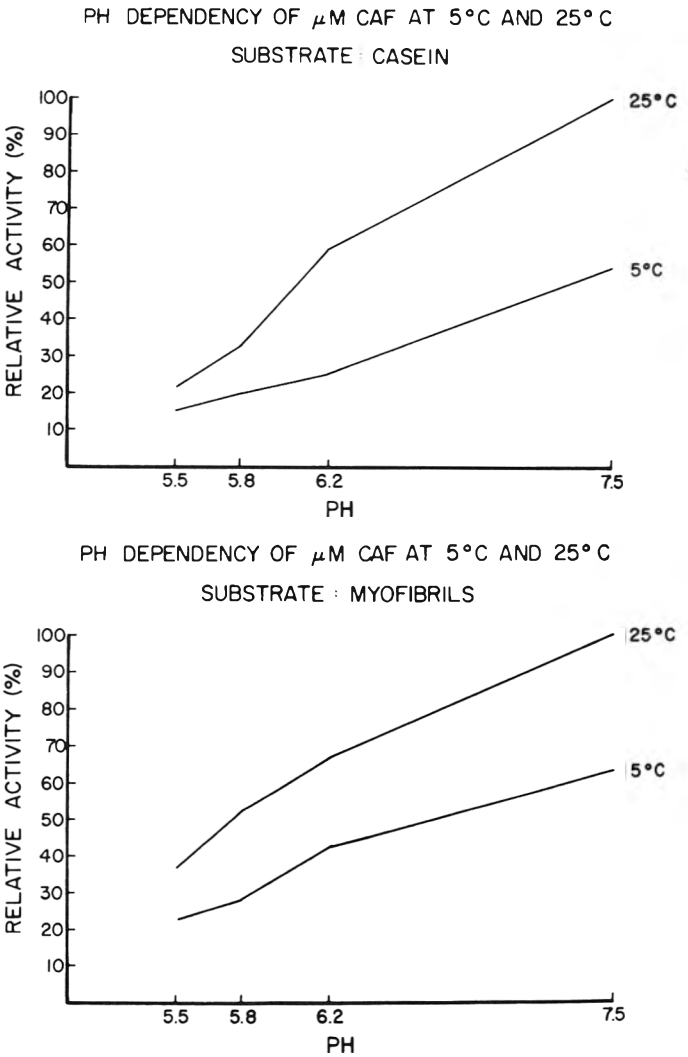

Fig. 1—Effect of pH on μm CAF activity at 5 and 25°C with casein or bovine skeletal myofibrils as substrate. Assay conditions: 100 mM KCl, 50 mM Tris-acetate, pH 7.5 (or stated pH), 10 mM 2-mercaptoethanol, 100 μM $CaCl_2$, 5 mg/mL of casein or myofibrils and 25 μg of purified μM CAF/mL, 90 min at 5 or 25°C.

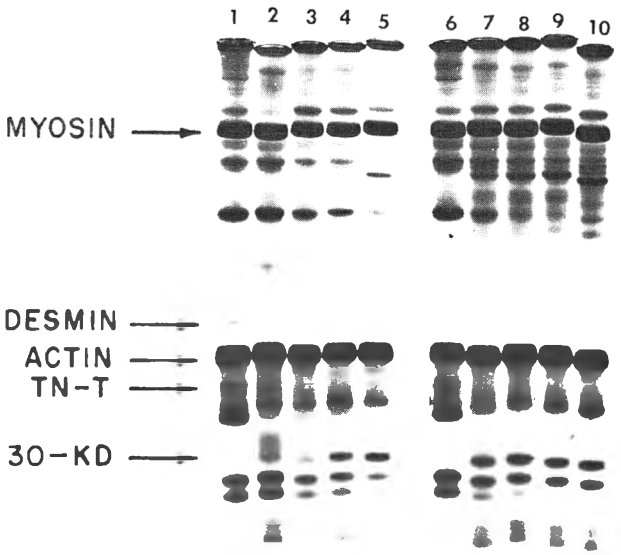

Fig. 2—SDS-PAGE of μM CAF-treated myofibrils isolated from at-death bovine sternomandibularis muscle under varying pH conditions at 5 and 25°C using 10% acrylamide with 0.1% bis-acrylamide (100:1) w/w at 400 mM Tris-glycine, pH 8.8, 5% glycerol and 0.10% SDS. Gels 1, 2, 3, 4, and 5 represent control, pH 5.5, 5.8, 6.2, and 7.5 at 5°C, respectively. Gels 6, 7, 8, 9, and 10 represent control, pH 5.5, 5.8, 6.2, and 7.5 at 25°C, respectively.

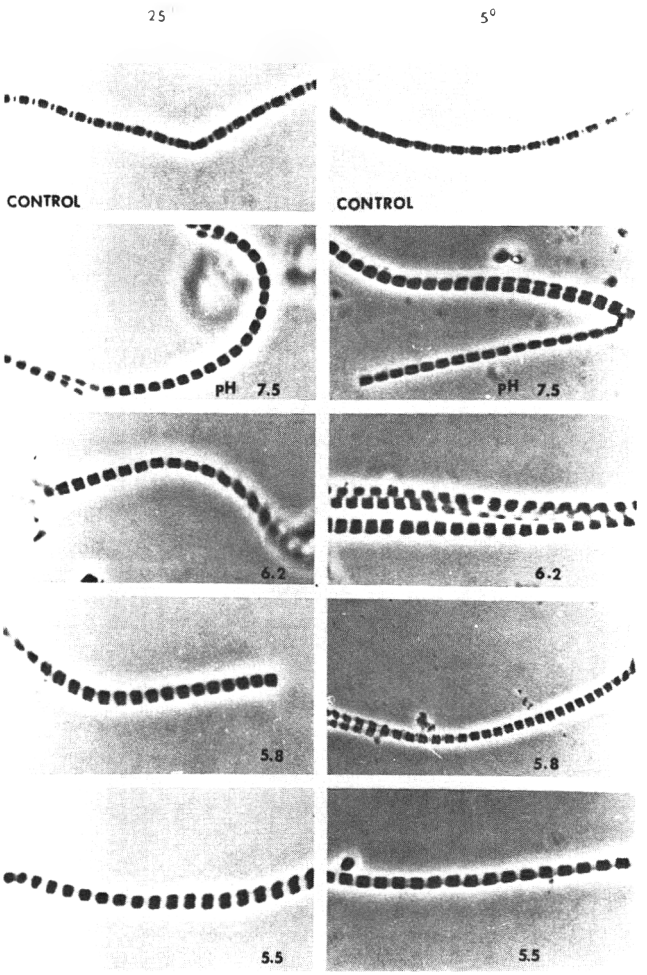

Fig. 3—Phase micrographs of μM CAF-treated myofibrils from at-death bovine sternomandibularis muscle (×2000). Note that the Z-disk-removing ability of μM CAF is not effected by pH at 25°C, while the Z-disk-removing ability decreases with decreasing pH at 5°C.

agreement with the conclusions of Penny (1980). As mentioned above, μM CAF at 5°C, retained 24 and 28% of its maximum activity at pH 5.5 and 5.8, respectively. The question arising at this point is whether this level of activity would be sufficient to bring about the changes occurring in muscle during postmortem aging. In order to answer this question, myofibrils were extracted from sternomandibolaris muscle immediately after slaughter and were incubated with chromatographically purified μM CAF and the effects were analyzed by SDS-PAGE, phase microscopy and electron microscopy.

Figure 2 shows the effect of μM CAF on myofibrillar protein under varying pH conditions at 5° and 25°C. Clearly, μM CAF can reproduce most of the changes observed during postmortem storage (pH 5.5–5.8 and 5°C), namely, (1) disappearance of desmin; (2) disappearance of troponin-T and appearance of a 30,000 dalton component; (3) removal of α-actinin, although incomplete, has begun; (4) no detectable effect on myosin and actin. A new band also appeared at about 130,000–150,000-dalton pH 6.2 and 7.5 at 5°C and at every pH at 25°C. Comparison of these results with the SDS-PAGE of myofibrils extracted from postmortem muscle (Koohmaraie et al., 1984a, b, c) strongly suggests that μM CAF could be responsible for the changes observed during postmortem storage. However, it appears that at pH 5.5–5.8 and 5°C these changes are incomplete (particularly the disappearance of troponin-T and appearance of a 30,000 dalton component). An inherent flaw in this experiment and any experiment of its kind is the inability to exactly mimic the behavior of postmortem muscle in an *in vitro* setting. Immediately after slaughter the pH and temperature of muscle are 7.0 and 37°C, respectively, and final conditions (pH 5.5–5.8 and 5°C) are achieved gradually over the first 24 hr of postmortem storage. Therefore, it is quite possible that the minor differences between electrophoretic patterns of CAF-treated myofibrils (at pH 5.5–5.8 and 5°C) and those of aged muscle (Koohmaraie et al., 1984a, b, c) are due to a gradual decrease in pH and temperature of muscle during postmortem storage. During this gradual change in pH and temperature, CAF would certainly be more active than at final conditions, pH 5.5–5.8 and 5°C (i.e., our experimental procedure).

Another similarity between postmortem aging and CAF treatment of myofibrils is the effect on desmin. Desmin appears to be the most susceptible protein to both these treatments, while there was a gradual increase in the degradation of troponin-T with increase in pH at 5°C, the removal of desmin is completed at pH 5.5 and 5°C. The same effect is observed during postmortem aging. By day three of postmortem storage, desmin is completely degraded while degradation of troponin-T is incomplete (Koohmaraie et al., 1984a).

Since the discovery of the degradation of troponin-T and simultaneous appearance of a 30,000 dalton component with postmortem aging (Olson et al., 1977), there has been considerable effort to correlate this change with the degree of meat tenderness (MacBride and Parrish, 1977; Penny and Dransfield, 1979). Indeed, Penny and Dransfield (1979) reported that this change was responsible for 60% of the variation in the toughness. These same workers also reported that in one of their samples (stored at 0°C at pH 6.3, conditions which cause muscle to cold-shorten) there was a loss of troponin-T without a corresponding increase in tenderness. One troublesome aspect of this hypothesis is a logistical one in that troponin-T is a constituent of the thin filaments of myofibrils and not of the Z-disks (Ebashi et al., 1968) and the first changes seen in myofibrils during postmortem storage in the loss of structural integrity of the Z-disks. On the other hand, desmin forms a proteinaceous network surrounding the Z-disks of each

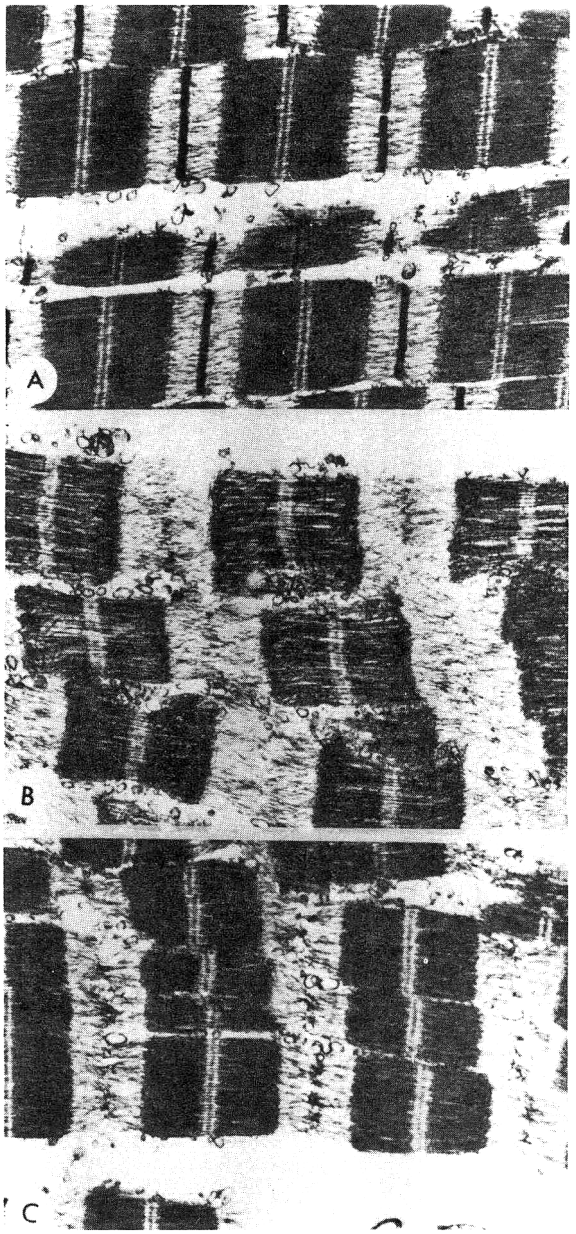

Fig. 4—Electron micrographs of μM CAF-treated bovine myofibrils. (a) Control at 25°C; (b) pH 7.5 at 25°C; (c) pH 6.2 at 25°C.

myofibril and is thought to interlock myofibrils at the Z-disk level (Robson et al., 1981). Because of the relative location of troponin-T versus desmin and also their degree of susceptibility to the conditions of postmortem storage and CAF treatment, it is proposed that the rate of the disappearance of desmin may be a better indicator of the degree of structural integrity of myofibrils at the Z-disk level and the subsequent increase in meat tenderness.

Figure 3 indicates the effect of pH on Z-disk-removing-ability of μM CAF at 5 and 25°C. These results clearly support those obtained by SDS-PAGE (Fig. 2). It demonstrates that μM CAF is clearly capable of completely removing Z-disks under all pH treatments at 25°C. However, its Z-disk-removing-ability gradually decreases with the decrease in pH at 5°C. At pH 5.5 and 5°C, Z-disks, although not completely removed, have a very faint appearance as compared to those of control myofibrils. As a result of postmortem storage, Z-disks are weakened, and this weakening of Z-disks is revealed by their faint appearance when examined by the light microscope (Davey and Gilbert, 1967, 1969). Based on the morphological changes

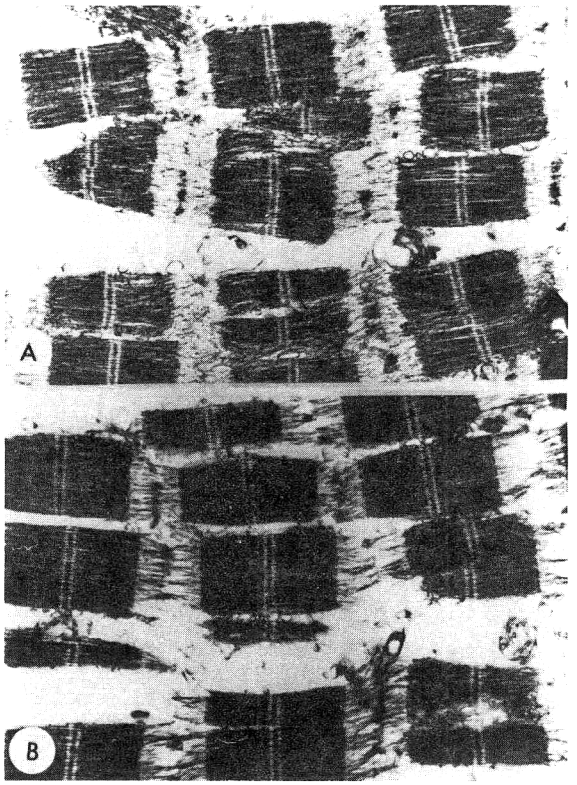

Fig. 5—Electron micrographs of μM CAF-treated bovine myofibrils. (a) pH 5.8 at 25°C; (b) pH 5.5 at 25°C.

seen in the Z-disks, therefore, again it can be concluded that μM CAF is capable of producing the changes observed in the muscle during postmortem storage. It is interesting to note that although CAF is not fully active at pH 5.5, 5.8, and 6.2 at 25°C, its Z-disk removing ability has not changed. Therefore, CAF does not need to be fully active to completely remove Z-disks.

Figures 4 through 7 show electron micrographs of control and μM CAF-treated myofibrils isolated from at-death bovine sternomandibularis muscle at varying pH and temperatures. These results clearly document the effect of pH on the Z-disk-removing ability of μM CAF at 5° or 25°C and confirm those results obtained by SDS-PAGE (Fig. 2) and phase microscopy (Fig. 3). As before, the Z-disk-removing ability of μM CAF was not affected by pH at 25°C as evidenced by complete removal of the Z-disk at all pH values examined. At conditions of interest (pH 5.5–5.8 at 5°C), although Z-disks are not completely removed, the Z-disks have clearly been affected. Based on the comparison between these results (pH 5.5–5.8 at 5°C) and those of aged myofibrils (Penny, 1980), it can be concluded that μM CAF is quite capable of reproducing observed changes in the muscle during postmortem aging.

In summary, the results of this present experiment demonstrated that although μM CAF was maximally active at pH 7.5, 24–28% of its maximum activity was retained at pH 5.5 to 5.8 and 5°C and, more importantly, this level of activity was sufficient to reproduce the changes observed during postmortem aging. Therefore, although the original questions which were raised concerning the involvement of CAF (the mM form) in postmortem aging may have been valid because of the pH and Ca^{++} concentrations required for the activity of that protease, it seems reasonable to suggest that with the identification of μM CAF these questions can no longer be of legitimate concern. We conclude that the low-Ca^{++}-requiring form of CAF is active at postmortem conditions and is capable of producing changes observed in the myofibrils during postmortem storage.

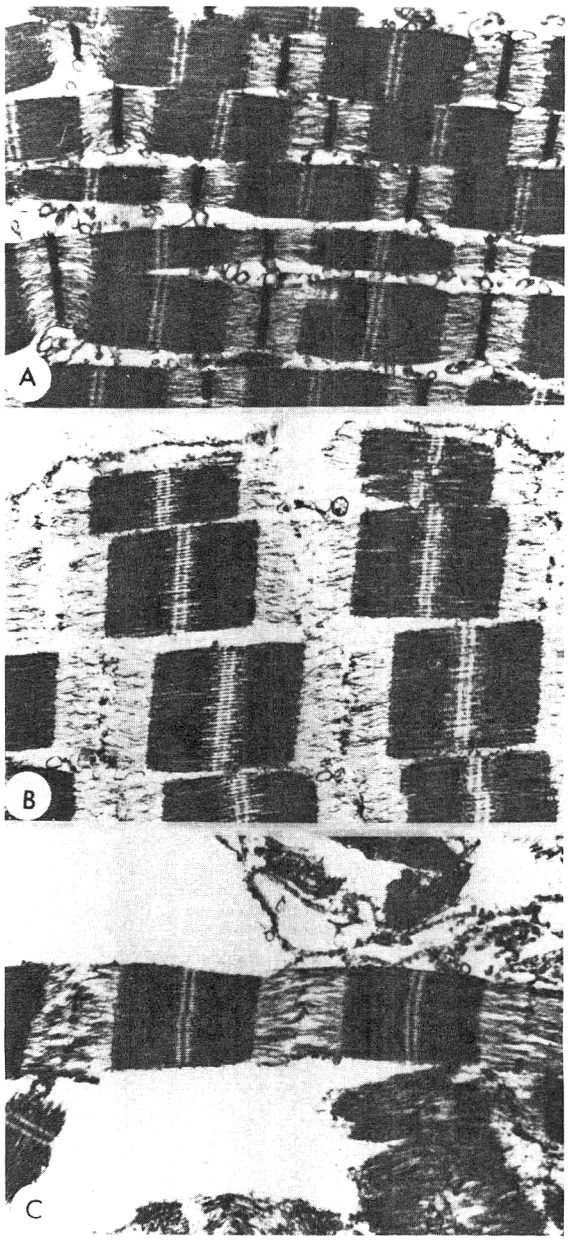

Fig. 6—Electron micrographs of μM CAF-treated bovine myofibrils. (a) Control at 5°; (b) pH 7.5 at 5°C; (c) pH 6.2 at 5°C.

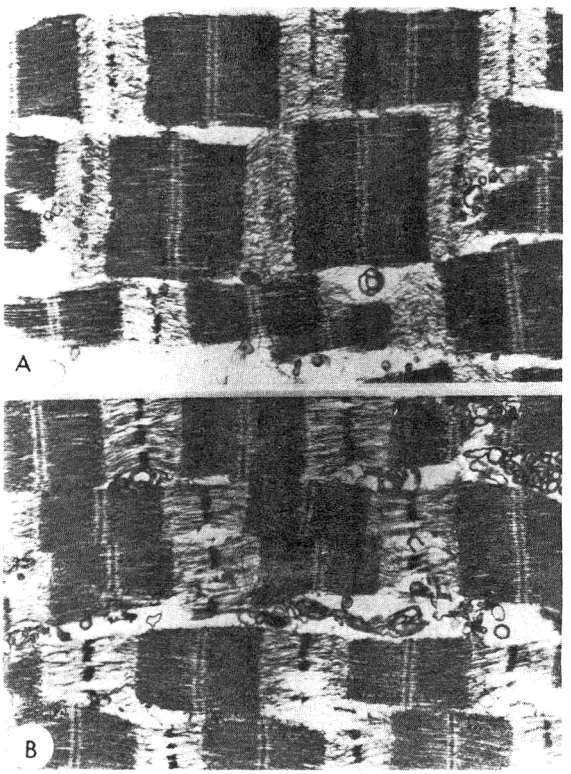

Fig. 7—Electron micrographs of μM CAF-treated bovine myofibrils. (a) pH 5.8 at 5°C; (b) pH 5.5 at 5°C.

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Effect of Gums on Low-Fat Meat Batters

E. A. FOEGEDING and S. R. RAMSEY

ABSTRACT

The effects of adding Iota-carrageenan, kappa-carrageenan, guar gum, locust bean gum, xanthan gum, methylcellulose, and a locust bean gum/kappa carrageenan mixture to low-fat, high moisture meat batters were investigated. The methylcellulose treatment showed an increase in weight losses between 60° and 70°C, while other treatments remained similar throughout heating. Xanthan gum and guar gum at 0.2% altered textural parameters as determined by texture profile analysis. Increasing the concentration of xanthan gum decreased batter hardness without affecting batter stability. Sensory evaluation indicated that low-fat frankfurters (11–12% fat) were as acceptable as control frankfurters (27% fat).

INTRODUCTION

FRANKFURTERS, wieners and bologna are processed meats that generally are catogorized as meat emulsions or batters and can contain a maximum of 30% fat. Current consumer interest in reduced calorie foods has led the American Meat Institute to petition USDA for a "light" standard of identity for cooked sausage (Anonymous, 1985). The standard would specify a maximum of 22.5% fat and a minimum of 11.5% protein.

Meat batters are formed by chopping meats, along with other ingredients, to form a coarse dispersion of mainly water, fat and protein. Thermal processing converts the highly viscous sol into a viscoelastic solid that can be viewed as a protein gel filled with fat particles. Since fat acts as a reservoir for flavor compounds and contributes to product texture, reducing the fat content may alter product quality. For example, lowering the fat content in wieners has been reported to increase toughness (Sofos and Allen, 1977; Paul and Foget, 1983). Thus, production of low-fat frankfurters requires altering currently used formulations and processes such that flavor and texture are as acceptable as current products. Sofos and Allen (1977) substituted textured soy protein for fat to produce an acceptable low-fat wiener.

Carbohydrate gums are used in the food industry to regulate viscosity and form gels. Wallingford and Labuza (1983) reported that xanthan gum was more effective than carrageenan, locust bean gum and low methoxy pectin in preventing water loss from a low-fat meat emulsion. Xanthan gum and carrageenan both were shown to stabilize the texture of frankfurters held in a vinegar pickle (Fox et al., 1983). These reports suggest that select gums are compatable with meat batters. The objective of this study was to evaluate the effect of replacing fat with water or water-gum suspensions on the cooking stability, textural and sensory properties of meat batters.

MATERIALS & METHODS

FRESH PORK HAMS, Boston butts, backfat and boneless beef (90% lean) were obtained from local processors. Hams and Boston butts were boned and trimmed of excessive fat. Individual meats were separately ground in sequence through plates with orifice diameters of 15 and 4 mm. Moisture, fat and protein contents were determined by the methods of AOAC (AOAC, 1980). Meats were wrapped with freezer paper (Marlon frozen food wrap) in batch-sized portions, frozen and held at −29°C. All meats were tempered at 2°C for 14 ± 1 hr prior to use.

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Iota-carrageenan (IC) (Viscarin® SD-389) and kappa-carrageenan (KC) (SeaKem® CM-611) were obtained from FMC Corporation (Marine Colloids Division, Springfield, NJ). Guar gum (GG) and locust bean gum (LB) were provided by Henkel Corporation (Minneapolis, MN), xanthan gum (XG) from Kelco, (Rahway, NJ) and methylcellulose (MC) (A4M Premium) from The Dow Chemical Company (Coral Gables, FL). Food grade salt, glucose, sodium nitrite and sodium erythrobate were used. Frankfurter seasoning was obtained from A. C. Legg Packing Co. Inc. (Birmingham, AL).

Experiment 1

All gums (IC, KC, GG, LB, MC, XG) were compared by substitution at 0.2% of the raw batter weight. In the kappa-carrageenan plus locust bean gum (KLB) treatment, 0.1% of each gum was used. The formulations for control-fat (CF), control-water (CW) and gum treatments are shown in Table 1. The ratio of protein from beef and pork was fixed at 1:1.5 respectively. A 2% suspension/gel of each gum was prepared and stored at 2°C prior to use. Batters were made by initially chopping beef trim, ground ham, one-half the ice and salt for 4 min in a bowl chopper (Hobart, Model 84142). Batter temperature was checked, then seasoning, nitrite, erythrobate, fat, remaining ice and gum suspension/gel were added and chopped an additional 4 min. Final batter temperature did not exceed 10°C. Batters were enclosed in plastic bags (Mobil Chemical PM1-6201, Covington, GA), held under vacuum to remove air pockets, then placed in a stuffing machine (Vogt Ideal 9, Germany). Approximately 30g of batter were extruded into weighed and stoppered polycarbonate tubes (25 mm internal diameter), re-weighed, then covered with aluminum foil and placed in a 20°C water bath. Batters were heated by a linear heating schedule of 0.6°C/min that was controlled by an Exacal EX 100 heater connected to an ETP-3 temperature programmer (Neslab, Portsmouth, NH). Triplicate samples were removed when batter temperatures reached 40°, 50°, and 60°C and six samples were removed at 70°C. Samples were cooled to 40°C. Cook-out liquid was drained into a weighed graduated centrifuge tube and batter plugs were removed.

Three batters heated to 70°C were held at 2°C for 24 hr, placed into water that had been heated to a boil, then removed from the heater

Table 1—Batter formulations

Ingredient/ component	Control-fat	Control-water	Gum treatments
Experiment 1			
Water	56.5%	72.0%	71.8%
Fat	25.5	10.0	10.0
Gum	0	0	0.2
Protein	13.0	13.0	13.0
NaCl	2.35	2.35	2.35
Glucose	2.00	2.00	2.00
Seasoning	0.60	0.60	0.60
Nitrite/ Erythrobate	0.05	0.05	0.05
Experiment 3			
Backfat	2188 g	259 g	259 g
Ice	807	2883	2883
Water	0	1200	1140
Gum	0	0	60
Nitrite	1.65	1.36	1.36
Erythrobate	4.66	4.66	4.66
Beef	3451	3451	3451
Pork	5166	5166	5166
Salt	282	282	282
Glucose	240	240	240
Seasoning	72	72	72

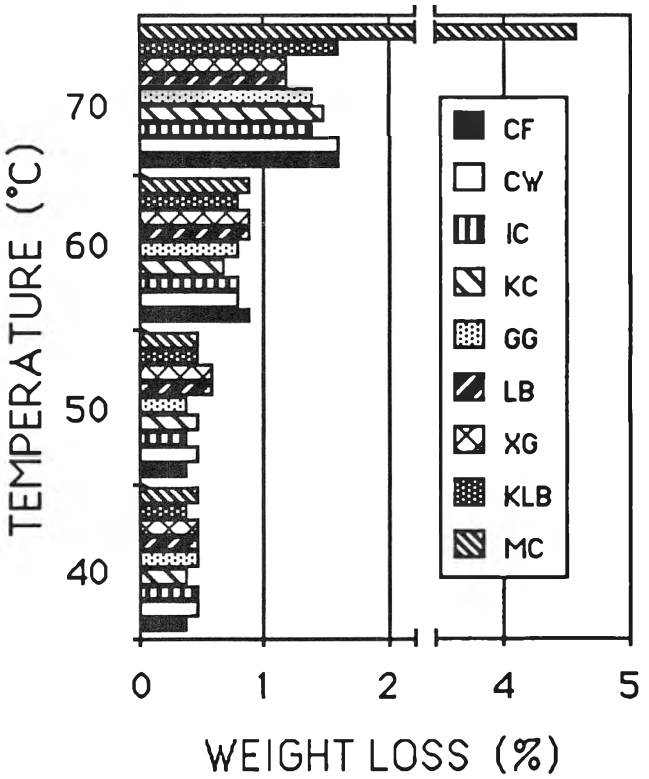

Fig. 1—Effect of temperature on weight loss of batters during heating. All gums were added at a level of 0.2%. CF = control-fat, CW = control-water, IC = iota carrageenan, KC = kappa carrageenan, GG = guar gum, LB = locust bean gum, XG = xantham gum, KLB = kappa carrageenan/locust bean gum and MC = methylcellulose.

Table 2—Effect of gums on textural parameters[a]

Treatment	Force to fracture (N)	Hardness (N)	Second-cycle hardness (N)
Control-Fat	73.4[f]	84.1[f]	65.5[f]
Control-Water	65.7[bf]	75.1[bcf]	60.4[bf]
Iota Carrageenan	69.0[bf]	77.2[bcf]	62.6[bf]
Kappa Carrageenan	62.4[bcf]	81.2[bf]	65.3[f]
Locust Bean Gum	69.4[bf]	72.7[bc]	58.6[bf]
Methylcellulose	59.5[bc]	75.4[bcf]	61.6[bf]
Kappa Carrageenan/ Locust Bean Gum	53.6[c]	66.4[cd]	53.8[bc]
Guar Gum	41.4[d]	59.0[de]	48.0[cd]
Xanthan Gum	36.4[d]	51.0[e]	39.6[d]

[a] Composite means for batters heated to 40°, 50°, 60°, and 70°C.
[b-f] Treatments with similar letters in columns are not significantly different (P>0.05).

Table 3—Mass balance of frankfurters[a]

Treatment		Moisture	Fat	Protein	Other
CF	raw	56.87 lb	23.23 lb	13.77 lbs	6.13 lbs
	cooked	44.71	23.41	13.77	4.97
CW	raw	73.12	9.51	11.51	5.86
	cooked	52.82	9.65	12.00	4.84
IC	raw	72.77	10.10	11.63	5.50
	cooked	54.36	9.92	11.61	4.93
KC	raw	73.61	9.23	11.71	5.45
	cooked	55.85	9.42	11.93	5.19

[a] Data are the average (three replications) values for weight in and out of thermal-processing, normalized to 100 lbs of raw batter.
[b] CF = control-fat, CW = control-water, IC = iota carrageenan and KC = kappa carrageenan.

and steeped for 7 min to simulate consumer cooking. All cooked batters were cooled to room temperature prior to textural analysis.

Textural characteristics of batters were determined using an Instron

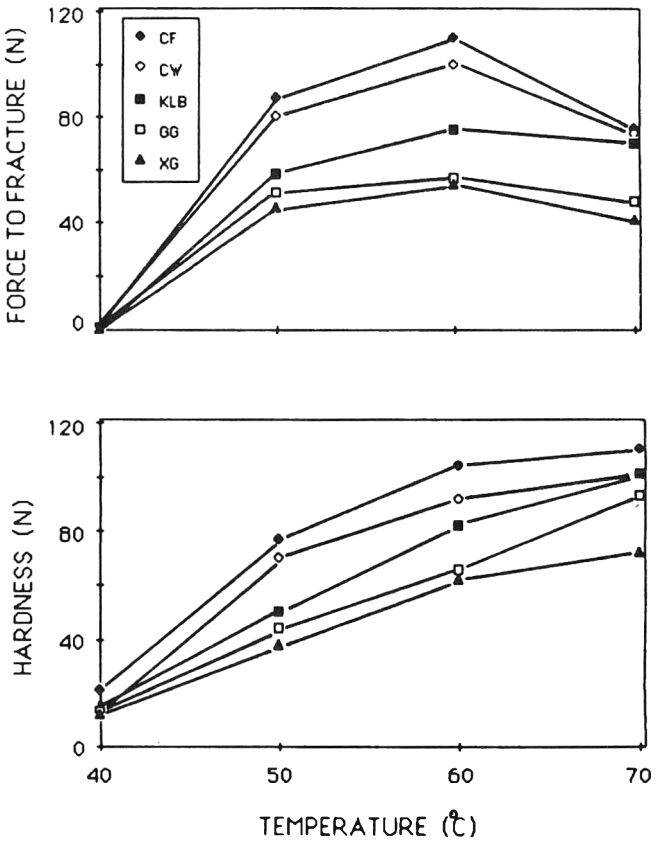

Fig. 2—Effect of heating on force to fracture and hardness. All gums were added at a level of 0.2%. CF = control-fat, CW = control-water, GG = guar gum, XG = xanthan gum and KLB = kappa carrageenan/locust bean gum.

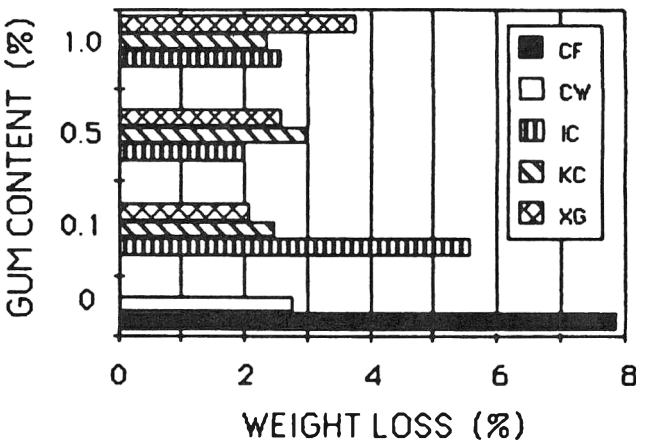

Fig. 3—Effect of gum concentration on batter weight loss. All batters were heated to 70°C. CF = control-fat, CW = control-water, IC = iota carrageenan, KC = kappa carrageenan, XG = xanthan gum.

Universal Testing Machine (Model 1130) and the Instron texture profile analysis method as described by Bourne (1978). After cooling to room temperature, four 13 mm long cylindrical samples, selected among the three plugs, were evaluated for each sampling. Batters were twice longitudinally compressed to 25% of original height at a cross-head speed of 100 mm/min. Textural parameters of force to fracture (FF), hardness (H1) and second-cycle hardness (H2) were measured from force-time curves. Springiness was calculated as the percentage of initial height recovered after the first compression.

Experiment 2

Frozen Boston butts and boneless beef were thawed at 2.2°C for 48 hr, then frozen and stored at −29°C for 16–23 days, until used.

Table 4—Held Moisture, Moisture, Fat, Protein, Sensory and Texture Analysis of Frankfurters

Treatment	Held[d] moisture	Moisture (%)	Fat (%)	Protein (%)	Panel score[e]	FF	H1 (N)	H2[f]
CF[g]	2.4[a]	51.4[a]	26.9[a]	16.0[a]	6.3[a]	135[a]	174[a]	143[a]
CW	2.7[ab]	66.6[b]	12.2[b]	15.1[b]	6.4[a]	101[bc]	152[bc]	114[bc]
IC	3.0[b]	67.3[b]	12.3[b]	14.4[b]	6.5[a]	84[c]	130[c]	96[c]
KC	2.9[b]	67.8[b]	11.4[b]	14.5[b]	6.1[a]	116[ab]	162[ab]	127[ab]

[a-c] Means within columns with similar superscripts are not significantly different (P>0.05).
[d] Values are reported as a weight ratio of water to protein.
[e] Hedonic scale was 9 = like extremely; 1 = dislike extremely.
[f] FF = force to fracture, H1 = hardness and H2 = second-cycle hardness.
[g] CF = control-fat, CW = control-water, IC = iota carrageenan and KC = kappa carrageenan.

The thaw-freeze cycle was used to decrease functional quality, thus increasing the potential for cooking losses. CF and CW treatments were manufactured as in experiment 1. Gum treatments were IC, KC and XG added individually at levels of 0.1, 0.5, and 1%. Batters containing gums were formulated and manufactured as in experiment 1, with water being exchanged for additional gum. Batters were cooked to 70°C and evaluated as described in experiment 1. No samples were removed prior to 70°C.

Experiment 3

Frankfurters were manufactured from Boston butts, backfat and boneless beef according to the formulations listed in Table 1. Beef, salt and one-half the ice were chopped in a Hobart VCM-40 chopper at slow speed for 3 min. The remaining ingredients were added and the mixture was chopped for 1 min at slow speed. Final chopping was at high speed until 15 ± 1°C for 2 min was achieved. Chopping was stopped at 1 min intervals to scrape the meat off the side of the bowl and monitor meat temperature. The batter was placed in a Vemag (Robert Reiser Co., Inc., Boston, MA) and vacuum stuffed into 26 mm No Jax casing (Union Carbide Corp., Chicago, IL). The linked (13.3mm) batters were processed in a Dry-Sys smokehouse by a stepwise heating schedule (relative humidity of 29–31%) until an internal temperature of 71°C was achieved. The cooked frankfurters were showered with cold water until they cooled to an internal temperature of 38°C and held at 4.5°C overnight. The frankfurters were peeled, vacuum packaged (clear std gauge, P850, Cryovac pouches, W.R. Grace and Co., Duncan, SC) and held at 4.5°C for further analysis. Raw and cooked batters were analyzed for moisture, fat and protein (AOAC, 1980); in addition, total raw and cooked weights were recorded and used for mass balance calculations. Three treatments, randomly assigned, were manufactured each day by processing.

Preliminary triangle test results indicated that there were detectable differences among normal and low-fat frankfurters. Therefore, an untrained panel consisting of 34 members was used to evaluate frankfurters on a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely). The panel contained 12 male and 22 female members ranging in age from 20 to 60 years. Frankfurters were prepared by holding in boiled water for 7 min. Samples (one-half frankfurter) were held at 57°C prior to being placed in randomly coded (three digit) polystyrene cups and presented in a randomized order to panelists. At each evaluation three samples were tasted in partitioned booths under red lighting. Panelists were asked to rinse with room temperature water between samples.

A modified method of Jauregui et al. (1981) was used to determine the water-holding properties of frankfurters. Two pieces of Whatman #3 (9.0 cm) and one piece of Whatman #50 (7.0 cm) filter paper were used to form a thimble with the Whatman #50 on the inside. Internal cores, 13 mm in diameter, were removed from frankfurters immediately prior to sampling. Samples weighing 1.5 ± 0.1g were cut from the cores and placed into pre-weighed thimbles then re-weighed. After centrifugation at 30,600 × *g* for 15 min the samples were removed from the thimbles and the thimbles were weighed to determine moisture released. Frankfurters were analyzed for moisture (AOAC, 1980), so that the amount of free and held water could be calculated. Instron texture profile analysis was run as described in experiment 1.

Statistical analysis

Experiments 1 and 3 had three replications and experiment 2 had four replications. All data were analyzed by analysis of variance using the General Linear Model procedure (SAS Institute Inc., Cary, NC). Waller-Duncan K-ratio t-test was used to test differences among means.

RESULTS & DISCUSSION

THE WEIGHT LOST by batters during heating in experiment 1 is shown in Fig. 1. The fluid released during cooking never developed a lipid layer, thus the amount of fat released was minimal. Methylcellulose was the only treatment to have a significant effect (P<0.05) on yield. The methylcellulose used in this study formed a firm gel at 50–55°C, while the main effect due to methylcellulose did not occur until 60–70°C. The molecular basis for the observed effect of methylcellulose was not investigated. Wallingford and Labuza (1983) added gums to meat emulsions at 0.2% and found that xanthan gum was more effective in holding water than locust bean gum, low methoxy pectin and carrageenan. While our results appear to not support their findings, a direct comparison between studies is difficult because of variations in formulations, ingredients and techniques.

Since no major losses occurred during heating, excluding the methylcellulose treatment, the batters were quite similar in composition when textural parameters were evaluated. Force to fracture (FF), hardness (H1) and second-cycle hardness (H2) were all significantly (P<0.05) influenced by treatment (Table 2). Second-cycle hardness was similar to H1 in trends due to treatment and temperature but was lower in magnitude.

The general trend in textural parameters among treatments was that CF, CW, IC, KC and LB were similar but different from the GG, XG and KLB treatments. In Fig. 2 it can be seen that the KLB treatment achieved FF and H1 values similar to CF and CW at 70°C, while XG never became as resistant to force as the controls. Holding batters in water that had been heated to boil, simulating consumer heating, failed to have a significant effect on texture.

The IC and KC treatments were most similar to the controls while XG differed greatly from controls (Table 2). To further investigate their effects, IC, KC and XG were added at levels of 0.1, 0.5 and 1% in experiment 2. The overall influence of gum concentration on weight losses was minimal, with CF and 0.1% IC treatments showing the greatest losses (Fig. 3). Control-fat was the only treatment with lipid losses, probably resulting from the thaw-freeze destabilization. Rupture of fat cells due to freezing is reported to decrease heat stability (Tinbergen and Olsman, 1979). Analysis of the textural data revealed that FF, H1, H2 and springiness were significantly affected (P<0.01) by both treatment and concentration (Fig. 4). As was seen in experiment 1, H2 followed treatment and concentration trends similar to H1 but lower in magnitude. The springiness of KC and IC treatments were relatively unaffected by gum content, contrasted to the major decrease in springiness due to an increase in xanthan gum (Fig. 4). The negative effect of xanthan gum on texture also was seen in FF and H1 values. Batters containing 1% xanthan gum heated to 70°C had textural properties quite similar to unheated batters or batters heated to 40°C, prior to any major thermally induced textural changes. It is interesting to note that on a weight loss basis, the XG treatments were quite favorable and similar to IC and KC treatments, whereas the textural analysis revealed major differences among treatments. Similar findings have been reported by Whiting (1984) who found that xanthan gum added at 0.1 or

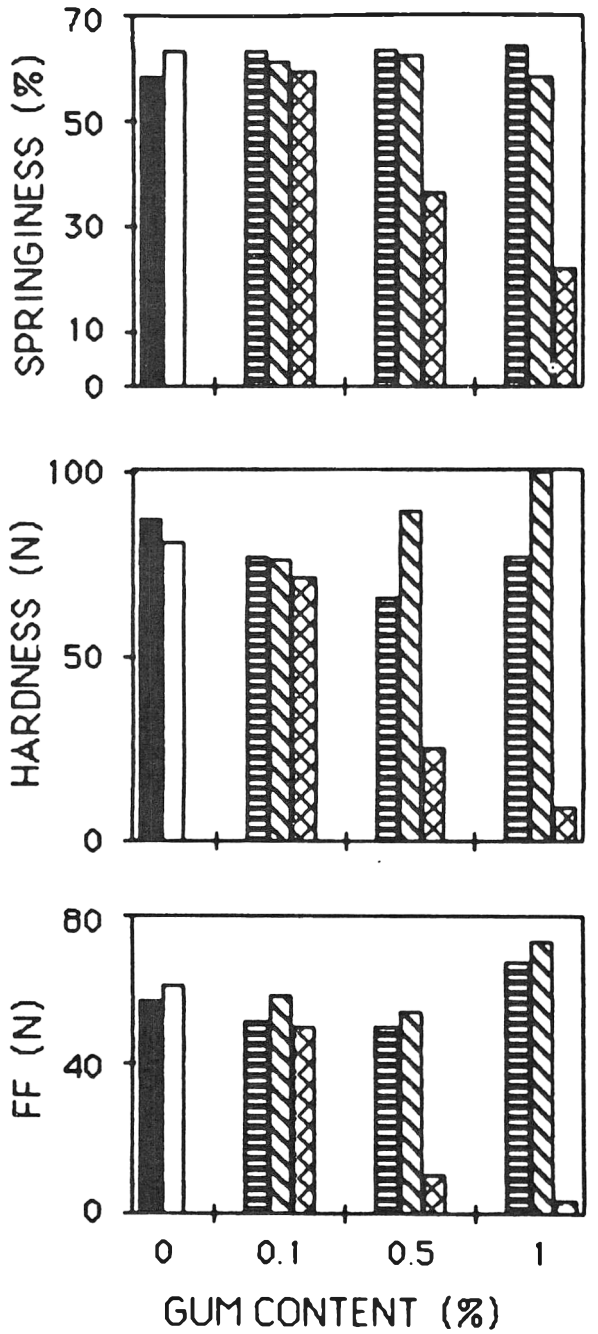

Fig. 4—Effect of gum concentration on textural properties. Treatments are: CF (solid black) = control-fat, CW (solid white) = control-water, IC (horizontal lines) = iota carrageenan, KC (diagonal lines) = kappa carrageenan and XG (crossed lines) = xanthan gum.

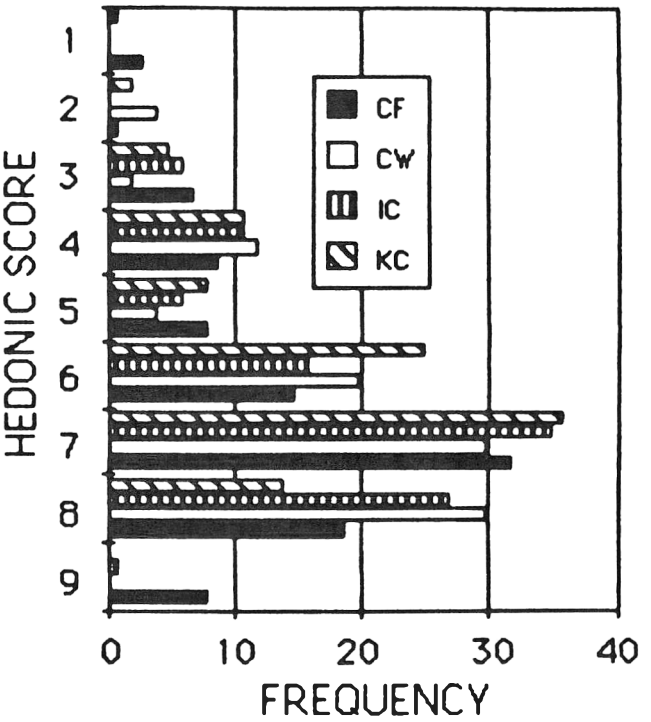

Fig. 5—Distribution of hedonic scores (9 = like extremely; 1 = dislike extremely) for frankfurters. Frequency indicates the number of times a hedonic score was given during 102 evaluations. CF = control-fat, CW = control-water, IC = iota carrageenan, KC = kappa carrageenan.

0.3% decreased cooking losses and gel strength. Thus, evaluating an ingredient solely on stability or textural parameters may lead one to false conclusions about the benefit of an ingredient. Consumer cooking (holding in boiled water for 7 min) had a significant effect ($P<0.01$) on H1, H2 and batter diameter. In all treatments, cooking increased H1 and H2 values and decreased batter diameter (ranging from 0.1 to 0.5 mm).

In both experiments 1 and 2, the meat batters were heated in covered polycarbonate tubes, thus removing any evaporative driving force for moisture loss. To determine how low-fat formulations would be affected by typical frankfurter processing conditions, treatments of KC, IC, CW and CF were manufactured using a typical frankfurter heat-processing schedule. The total weight of fat, water and protein in raw and cooked batters was used to calculate a mass balance of cooking losses (Table 3). Cooked batters were not analyzed for salt, seasoning, gums, glucose or other curing ingredients; thus the weight lost due to these ingredients is reflected in the "other" category. The slight increases in fat and protein for several treatments are due to inherent variation in the analytical methods. The main weight loss for all treatments was due to water. It also appeared that some of the non-meat solids were lost, probably because they were dissolved in the aqueous cook-out. These results indicated that stable batters were formed, i.e. no major fat loss during thermal processing, and suggests that the evaporative driving force and fat-to-protein ratio were the main regulators of moisture losses during cooking. The effects of fat-to-protein ratio and relative humidity on water losses in frankfurters were reported by Mittal and Blaisdell (1983). Their results indicated that moisture loss rate increased as fat-to-protein ratio decreased. Trends observed in this study support their results. Also reported by Mittal and Blaisdell (1983) was that increasing smokehouse relative humidity from 41 to 60% increased moisture losses while an increase from 60 to 87% decreased losses, suggesting that a high relative humidity, i.e. >60%, is required to decrease water losses.

There was no significant difference among the low-fat treatments for moisture, fat or protein content (Table 4). Subjecting the cooked batters to a mild centrifugal force gave an indication of how well the moisture was held in the batters. Generally speaking, water holding ability of meat is related to protein content so the amount of water held in the system after centrifugation was expressed as a ratio to the protein content (Table 4). The increase in held moisture seen in both carrageenan treatments could be due to a gum-water interaction or a gum-protein-water interaction. Whatever the mechanism, the carrageenans appeared to increase the water holding ability of frankfurters.

The results of textural and sensory analyses of frankfurters are shown in Table 4. There were significant differences

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Effect of Slaughter-Dressing, Fabrication and Storage Conditions on the Microbiological and Sensory Characteristics of Vacuum-Packaged Beef Steaks

S.K. CHANDRAN, J.W. SAVELL, D.B. GRIFFIN, and C. VANDERZANT

ABSTRACT

Aerobic plate counts (APC) of steaks from carcasses processed under strict sanitary slaughter-dressing conditions did not differ ($P>0.05$) from those of steaks from carcasses handled under conventional conditions. However, the former had frequently less off-odor than the latter. Steaks obtained under strict sanitary fabrication procedures had lower ($P<0.05$) APC and often less off-odor than conventionally fabricated steaks. Steaks stored at 0–1°C in the dark usually had less off-odor than steaks stored in a retail display case at 2–5°C. The microflora of conventionally fabricated steaks had a greater percentage of *Pseudomonas* and *Moraxella-Acinetobacter* spp. than that of steaks fabricated under strict sanitary conditions.

INTRODUCTION

TO OBTAIN optimum shelf-life of refrigerated fresh red meat, it is necessary to limit microbial contamination and growth on the carcass and to restrict further contamination and growth during fabrication of carcasses into primal, subprimal and retail cuts. Numerous studies have reported on the sources of carcass contamination such as the hide, hooves, hair, intestinal contents, contact with contaminated utensils, equipment, hands and garments of personnel and faulty slaughtering-dressing practices, particularly during evisceration. Classical reports on this subject are those of Empey and Scott (1939) and Ayres (1955). There are, however, differences of opinion about the extent to which these various sources of contamination contribute to the microbiological condition of a carcass (Ingram and Roberts, 1976; Roberts, 1980). It is generally accepted that the microbiological condition of utensils and equipment and the degree of sanitation applied by workers during slaughter-dressing operations have an important effect on the microbiological condition of a carcass. According to Hess and Lott (1970), workers' hands and tools are key sources of carcass contamination. Surface contamination (cold-tolerant bacteria and *Enterobacteriaceae*) of freshly slaughtered beef carcasses prepared with careful cleaning of hands and knives was lower than that of carcasses prepared with routine practices. Smulders and Woolthuis (1983) reported that APC's of veal cuts (*longissimus*) produced under "strict hygienic" conditions were 1 $\log_{10}$ lower ($P<0.05$) than those of cuts produced under "hygienic conditions."

It is generally accepted that shelf-life of refrigerated fresh meats at the retail level is related to the microbiological condition of the meat as received at retail, retail handling and temperature of storage. According to Greer et al. (1983), the case-life of retail beef steaks was related to the psychrotrophic bacterial contamination on wholesale ribs and steaks and the level of retail processing sanitation. Retail case temperature also is an important factor in shelf-life; as the temperature of steaks was increased 1°C within the range 2–12°C, there was a 0.63-day decrease in the retail case-life of beef (Greer, 1981; Greer and Jeremiah, 1981). The objective of this study was to compare the microbiological and sensory characteristics of vacuum-packaged steaks obtained with (1) two levels of sanitation during (a) slaughter-dressing and (b) fabrication procedures; (2) storage of steaks in a retail case at 2–5°C vs in the dark at 0–1°C.

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Table 1—Mean aerobic plate counts ($\log_{10}$/cm²) of vacuum-packaged steaks as influenced by component treatment groups of the slaughter-dressing, fabrication and storage procedures[a,b]

Days of storage	Slaughter-dressing[c]		Fabrication[c]		Storage[c]	
	Conventional	Strict sanitary	Conventional	Strict sanitary	Retail display	In the dark
0	2.34	2.04	3.25	1.13	2.19	2.19
7	3.48	3.66	4.79	2.01	3.95	3.22
14	4.31	4.71	5.83	3.19	4.95	4.07
21	5.27	5.67	6.45	4.50	5.65	5.29
28	6.18	6.07	6.77	5.53	6.21	6.01
35	6.75	6.46	7.11	6.03	6.51	6.75
42	6.67	6.75	7.06	6.36	6.33	7.09

[a] Three steers were used for both the conventional and strict sanitary slaughter-dressing procedures.
[b] The initial number of steaks (day 0) for each treatment was 234; the bacterial counts reported at each of the storage intervals are the means of 18 to 36 steaks.
[c] Means in the same row (days of storage) pertaining to the same procedure (slaughter-dressing, fabrication or storage) underscored by a common line do not differ ($P>0.05$).

Table 2—Mean off-odor scores of vacuum-packaged steaks as influenced by component treatment groups of the slaughter-dressing, fabrication and storage procedures[a,b]

Days of storage	Slaughter-dressing[c]		Fabrication[c]		Storage[c]	
	Conventional	Strict sanitary	Conventional	Strict sanitary	Retail display	In the dark
1						
7	9.45	9.68	9.56	9.57	9.47	9.66
14	8.23	8.81	8.19	8.85	8.16	8.89
21	7.66	7.77	7.32	8.10	7.21	8.21
28	7.10	7.25	6.83	7.49	6.66	7.88
35	6.43	6.60	6.32	6.75	5.88	7.25
42	5.20	6.50	5.94	5.76	5.07	6.63

[a] Off-odors were not determined for samples at day 1 of storage.
[b] Scores reported are the means of 18 to 36 steaks. Means based on a 10-point scale (10 = no off-odor; 1 = abundant off-odor).
[c] Means in the same row (days of storage) pertaining to the same procedure (slaughter-dressing, fabrication or storage) underscored by a common line do not differ ($P>0.05$).

MATERIALS & METHODS

Beef carcasses

Two beef carcasses (grain-fed, U.S. Good or Choice, 450-500 kg steers), one slaughtered and dressed by conventional procedures and the other by strict sanitary procedures, were obtained from the Meat Science and Technology Center, a teaching, research and extension facility at Texas A&M University. This procedure was replicated in

Table 3—Mean lean color scores of vacuum-packaged steaks as influenced by component treatment groups of the slaughter-dressing, fabrication and storage procedures[a]

Days of storage	Slaughter-dressing[b]		Fabrication[b]		Storage[b]	
	Conventional	Strict sanitary	Conventional	Strict sanitary	Retail display	In the dark
1	11.59	11.08	11.21	11.46	11.37	11.29
7	12.42	11.99	12.04	12.37	12.23	12.19
14	12.59	11.96	12.20	12.35	12.33	12.21
21	12.30	11.81	12.06	12.04	12.18	11.93
28	12.47	11.97	12.40	11.97	12.35	12.03
35	12.36	12.46	12.69	12.11	12.44	12.38
42	12.44	11.44	12.41	11.00	11.33	12.61

[a] The number of steaks for which mean scores are reported ranged from 216 at day 1 to 18 at day 42. Means based on a 15-point scale (15 = bright purple red; 1 = extremely dark brown).

[b] Means in the same row (days of storage) pertaining to the same procedure (slaughter-dressing, fabrication or storage) underscored by a common line do not differ ($P>0.05$).

three separate and independent trials (n = 6 beef carcasses). Beef slaughter at this facility is an on-the-rail, gravity-flow procedure in which workers utilize both hand-knives and air-operated dehiders to prepare the animal for hide removal by the use of a commercial (caudal or "up") hide puller. Except for number of animals handled, equipment and processing procedures at this facility are equivalent to those found in commercial operations. Conventional slaughter and dressing involved normal sanitary efforts with respect to handling of the carcass, equipment and utensils (equipment, workmen and slaughter procedures were governed by applicable meat inspection regulations). Strict sanitary slaughter and dressing procedures involved frequent rinsing and sanitation (tap water, 80°C) of knives, use of clean garments and sterile, disposable gloves, frequent washing and rinsing of hands and arms with soap and water and careful handling of the carcass to prevent cross contamination. Beef carcasses were fabricated after completion of rigor mortis (approximately 24 hr after slaughter) after being held at 0°-1°C in a chilling room equipped with high-velocity fans to promote rapid air movement.

Fabrication

Boneless strip loins were carefully removed from each side of the intact carcasses from both the strict sanitary and conventional slaughter-dressing treatments. They were placed on sanitary trays and transported to refrigerated cutting rooms (7°C). One strip loin from each carcass was fabricated using conventional procedures while the other strip loin was fabricated using strict sanitary procedures. Twenty steaks (1.27 cm thick) were cut from each loin. Each steak was then cut in half through the approximate center of the *longissimus dorsi* muscle. Thirty-nine of the 40 steaks from a loin were used per fabrication treatment.

The room for conventional fabrication procedures was in full production use during the experiment. For conventional fabrication, strips were cut midmorning and handled on tables where beef had been cut just before the experimental samples were fabricated. Condition of equipment, handling procedures and workmen were governed by meat inspection regulations. Strict sanitary fabrication was conducted in a separate, but comparable cutting room which was not used for commercial production at that time. Procedures included the use of clean garments and sterile disposable gloves, thoroughly cleaned and sanitized work surfaces, sterile plastic transport trays, and knives that were rinsed and then immersed in 95% ethanol and subsequently flamed before each cutting operation.

Packaging

All steaks (78 samples per animal, 156 samples per replicate) were vacuum-packaged in high-oxygen barrier film (Saran-coated polyethylene film, T.W. Kutter and Co.). The oxygen transmission rate of the film was 7.8 $cc/m^2/24$ hr at 23°C and 0% RH. The moisture transmission rate was 9.3 $cc/m^2/24$ hr at 38°C and at 90% RH. The steaks were packaged at an approximate vacuum of 29.5 mm of Hg in a chamber-type heat-seal system (Boss 6380, Bad Hamburg 6).

Storage

Three of the 39 steaks from each loin were used to determine the number and types of microorganisms at day 0. One-half of the remaining samples (18 steaks) from each side of the two carcasses was stored at 2–5°C under simulated retail display conditions in display cabinets (Tyler Model DM8) under fluorescent (GE "Natural") light (1614 lux). The others (18 steaks) were placed in cardboard boxes and stored in the dark at 0–1°C.

Sensory evaluation

All steaks were evaluated at weekly intervals by a three-member trained panel for surface discoloration (15 = 0% discoloration; 1 = 100% discoloration), lean color (15 = bright purple red; 1 = extremely dark brown) and overall appearance (15 = extremely desirable; 1 = extremely undesirable) as described by Griffin et al. (1982). Steaks were evaluated under simulated retail display conditions as described above. Steaks subjected to microbiological analysis were also evaluated at weekly intervals by the panel for off-odor (10 = no off-odor; 1 = abundant off-odor) immediately after opening of the packages. When all steaks from a single treatment became unacceptable in terms of sensory characteristics, they were removed from storage.

Microbiological analysis

At weekly intervals, the aerobic plate count (APC) was determined on three steaks randomly picked from each treatment. A 10-cm^2 piece (2 mm thick) was cut from the surface of each steak with sterile scalpels and placed in a sterile stomacher bag with 100 mL sterile 0.1% peptone (Difco). Following blending for 1 min, 0.1-mL volumes of appropriate decimal dilutions were plated on prepoured tryptic soy agar (Difco) plates. Plates were incubated at 25°C for 2 days. The distribution of microbial types in the microflora of the steaks was determined at day 0, at the end of the 42-day storage period, or when a sample became unacceptable in terms of sensory characteristics. Details of methods used to determine APC and percentage distribution of microbial types on countable plates are described by Vanderzant et al. (1982).

Table 4—Percentage distribution of microbial types on day 0 of storage of vacuum-packaged steaks obtained by conventional and strict sanitary slaughter-dressing and fabrication procedures

Microbial types	Percentage distribution of microbial flora			
	Conventional slaughter-dressing		Strict sanitary slaughter-dressing	
	Conventional fabrication	Strict sanitary fabrication	Conventional fabrication	Strict sanitary fabrication
Pseudomonas	66.8[a]	25.0[a]	56.0[a]	35.7[a]
Acinetobacter-Moraxella	12.7	3.5	1.2	
Staphylococcus	0.2	17.7	1.2	
Micrococcus	2.3	30.2	13.4	41.4
Yeasts	<0.1		11.1	
Brochothrix thermosphacta	4.7	1.7	6.2	6.2
Coryneform bacteria	0.7	16.7	1.7	16.7
Lactobacillus cellobiosus	3.2	3.1	8.7	
Lactobacillus coryneformis		2.1		
Lactobacillus plantarum	2.5		0.5	
Leuconostoc paramesenteroides	6.9			

[a] Percentages are expressed in terms of the total microbial flora on countable plates (means of nine steaks).

Table 5—Percentage distribution of microbial types on steaks obtained by conventional and strict sanitary slaughter-dressing procedures on day 42 of storage in retail display cases

	Conventional slaughter-dressing		Strict sanitary slaughter-dressing	
	Fabrication		Fabrication	
Microbial type	Conventional	Strict sanitary	Conventional	Strict sanitary
Pseudomonas	0.2[a]	16.8[b]		16.3[b]
Serratia liquefaciens	0.8			16.7
Brochothrix thermosphacta		17.6		
Micrococcus	0.6	11.3		
Leuconostoc mesenteroides		12.9	12.4[a]	2.4
Leuconostoc paramesenteroides	1.1		3.1	
Lactobacillus cellobiosus	55.8	22.4	50.9	23.6
Lactobacillus plantarum	41.5	19.0	33.6	41.0

[a,b] Percentages are expressed in terms of the total microbial flora on countable plates (a = means of three steaks, b = means of six steaks).

Statistical analysis

Differences in microbiological counts, and sensory evaluation scores of samples between treatments within slaughter-dressing, fabrication and storage procedures were examined for significance by analysis of variance (SAS, 1982). When significant ($P<0.05$) main effects were observed, mean separation was accomplished by the use of Duncan's multiple range test (Duncan, 1955).

RESULTS & DISCUSSION

DIFFERENCES in the level of sanitation as applied to the slaughter-dressing method did not significantly influence bacterial counts of vacuum-packaged steaks (Table 1). Steaks obtained from carcasses processed under strict sanitary slaughter-dressing procedures had less off-odor for three of the six storage intervals (Table 2), but lower lean color scores for six of the seven storage intervals (Table 3) than did steaks obtained from carcasses processed under conventional slaughter-dressing procedures. Effects of degree of sanitation during slaughter-dressing on overall appearance and surface discoloration scores were often not significant or inconsistent (data not shown in tabular form).

At all storage intervals, steaks obtained with the strict sanitary fabrication procedure had lower ($P<0.05$) bacterial counts than those obtained with the conventional fabrication procedure (Table 1). Differences in APC between steaks fabricated under conventional and strict sanitary conditions were approximately 2 $\log_{10}$ at the first four storage intervals (0, 7, 14, 21 days) and about 1 $\log_{10}$ at the next three storage intervals (28, 35, 42 d). Smulders and Woolthuis (1983) also reported significant decreases in bacterial counts of veal (*longissimus* cuts) when strict sanitary fabrication practices were applied. According to Greer et al. (1983), the case-life of retail beef steaks was related to the psychrotrophic bacterial content on the wholesale ribs and steaks and the degree of sanitation at the retail level. From a practical standpoint, case-life of steaks could be predicted by the following equation (Greer et al. 1983): case-life (days) = 3.97 − 0.19 (log bacteria/cm^2 on wholesale ribs) − 0.14 (log bacteria/cm^2 on meat processing equipment).

Steaks obtained with strict sanitary fabrication practices had less off-odor at three of the six storage intervals (14, 21, and 28 days) compared to steaks obtained with conventional fabrication practices (Table 2). This difference in off-odor is most likely related to the fact that bacterial counts of steaks fabricated under strict sanitary conditions were lower than those of steaks produced under conventional fabrication practices. Effects of variation in degree of sanitation during fabrication of steaks on lean color, overall appearance and surface discoloration scores were in most cases either not significant or inconsistent.

In most cases, method of storing steaks (retail display case at 2–5°C versus in the dark at 0–1°C) did not influence their bacterial counts. Counts of steaks stored in retail display cases for 7 and 14 days were higher ($P<0.05$) than those of comparable steaks stored in the dark (Table 1). Steaks stored in the dark had less off-odor at five of the six storage intervals than steaks stored in a retail display case (Table 2). Production of off-odors in steaks by microbial and tissue enzymes at 0–1°C (storage in the dark) may have been somewhat slower than on steaks stored at 2–5°C in retail display cases. Effect of method of storing steaks on lean color, overall appearance and surface discoloration scores was either not significant or inconsistent. Temperatures of 2–5°C are representative of optimum conditions under retail display; temperatures of 0°-1°C are comparable to optimum chilling conditions for meat during the wholesale storage phase.

At day 0, the microflora of steaks fabricated under conventional conditions (Table 4) had a higher percentage of typical gram-negative spoilage bacteria (*Pseudomonas, Moraxella-Acinetobacter*) than that of steaks obtained under strict sanitary fabrication procedures. The microflora of steaks fabricated under conventional conditions was dominated by *Pseudomonas* spp., whereas *Micrococcus* and *Pseudomonas* spp. were major parts (≥25%) of the microflora of steaks fabricated under strict sanitary conditions. Coryneform bacteria were more apparent in the microflora of steaks fabricated under strict sanitary conditions than in that of steaks produced under conventional practices. There appeared to be fewer lactic acid bacteria in the microflora of the steaks fabricated under strict sanitary conditions than in that of steaks fabricated under conventional conditions. No lactic acid bacteria were detected in the microflora of steaks obtained under strict sanitary conditions of slaughter-dressing and fabrication. The microflora of steaks stored for 42 days was dominated by lactic acid bacteria, particularly *Lactobacillus cellobiosus, Lactobacillus plantarum*, and *Leuconostoc mesenteroides* (Table 5, data shown only for steaks stored in retail display cases). The microflora of those steaks that were removed after 21, 28, and 35 days of storage because of unacceptable sensory properties, was in most cases dominated by *L. cellobiosus* and *L. plantarum* (data not shown in tables). The microflora of steaks obtained under conventional slaughter-dressing and fabrication procedures initially and after refrigerated storage was similar to that reported in other studies (Stringer et al., 1969; Vanderzant et al., 1982).

Conventional slaughter-dressing and fabrication procedures in this study refer to procedures representing "good manufacturing practices." Strict sanitary practices are those somewhat more elaborate than those usually applied in commercial operations. Procedures not consistent with "good manufacturing practices" were not applicable to this study. In summary, the data indicate that strict sanitary fabrication practices can (a) reduce total bacterial counts, (b) reduce the percentage of typical gram-negative spoilage bacteria in the microflora of steaks at day 0, and (c) reduce off-odor development of refrigerated vacuum-packaged steaks. The effect of strict sanitary slaughter-dressing and fabrication procedures on lean color, overall appearance and surface discoloration scores was not so clear.

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Prediction of Pork and Lamb Meat Quality Characteristics

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ABSTRACT

A two-factor central composite rotatable design and corresponding response surface analysis were successfully applied to data from pork and lamb loin roasts, using cooking temperature and endpoint temperature as the two independent variables. Heating rate (°C/min), evaporation loss, cooking time, total moisture, and chromaticity coordinate (z) were found to be significantly affected ($p \leq 0.10$) by different combinations of cooking temperatures and endpoint temperatures in pork and lamb. Additional significant variables for pork were total nitrogen, remaining protein fraction and sensory panel juiciness; and for lamb, heating rate (°C/g), drip loss, total cooking loss, expressible moisture index, total nitrogen, chromaticity coordinate (x), saturation index, and sensory panel doneness and color. Response surfaces were useful in evaluating results.

INTRODUCTION

THE RELATIONSHIP of pork and lamb quality characteristics to various heat treatments is important for optimum product quality and thus the success of the meat industry. Much early research focused on the influence of cooking and endpoint temperatures on various quality characteristics. Mackey and Oliver (1954) evaluated pork loin sampling methods for cooking tests, and Tuomy and Lechnir (1964) reported the effect of cooking temperature and time on the tenderness of pork. Holmes et al. (1966) evaluated the effect of internal temperature on eating quality of broiled chops. The need to improve lamb palatability is also considered important by the sheep industry (Lind et al., 1971). There is a renewed interest in lamb and mutton flavor components (Crouse, 1983; Cramer, 1983; Field et al., 1983; Vesely, 1973); however, little has been written about cooking temperature effects on lamb. In most studies on both meats, results found are unique to the specific experimental conditions under which the research was done.

The objective of this study was to investigate the ability of a central composite rotatable design (CCRD), using cooking temperature (CT) and endpoint temperature (ET) as the independent variables, to predict selected chemical, physical and sensory pork and lamb loin quality characteristics considered important by the industry, researcher, and consumer alike.

MATERIALS & METHODS

Samples

Paired pork loins (averaging 1088g, 2.3% total nitrogen, 74.2% total moisture, from 5.5–6 month old barrows and gilts) and paired lamb loins (averaging 424g, 3.2% total nitrogen, 71.7% total moisture) were from carcasses held after slaughter for 48 hr at 0°C. Loin roasts were removed between the second and 10th vertebrae (thus including the third and ninth). Left and right sides were individually wrapped in film-lined freezer wrap and pork was stored (3–4°C) for 3 to 7 days; roasting was completed within 7 days. Lamb samples were stored at −18°C until used, then defrosted 48 hr (4°C) before roasting.

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Statistical design

A two-factor Central Composite Rotatable Design (CCRD) (Zondagh, 1985) was chosen to allow maximum cooking and internal endpoint temperature coverage with the limited amount of sample available (Cochran and Cox, 1957). The independent (X) variables were cooking (oven) temperatures (CT) and internal endpoint temperatures (ET). The results obtained from the objective (chemical and physical) and sensory tests were the dependent (Y) variables or responses. Response surface analysis (RSA) was used to better understand the nature of the responses obtained with the 13 CT-ET combinations used on the pork or lamb roasts. Table 1 depicts the basic CCRD (Cochran and Cox, 1957) and the CT-ET combinations used. For statistical analysis, the level of significance was preestablished at $P \leq 0.10$.

Sample preparation

Each individual roast was placed on a wire rack in a foil-lined, aluminum roasting pan and baked in the center of a preheated electric oven. CT-ET combinations were randomly allocated to the raw pork and lamb roasts. One CCRD design replication was accomplished for pork; duplicates were possible for the lamb loin roasts, using the left sides versus right sides, and resulted in 26 observations. The roasted, excised longissimus dorsi muscle was used for the various physical, chemical and sensory tests (Fig. 1A and 1B).

The left pork and lamb loin roasts were used for evaluating total moisture, expressible moisture index, total nitrogen, and protein solubility, and the right loin roasts for sensory, Photovolt color and Warner-Bratzler (W-B) shear tests (Fig. 1A). The pork sensory test sampling is indicated in Fig. 1A, and sampling strategy for lamb sensory and shear tests is shown in Fig. 1B.

Testing methods

Cooking data. Internal temperatures during heating of the pork and lamb roasts were monitored at the center of the longissimus dorsi with a Leeds and Northrup W12 Temperature Recorder (Leeds and Northrup, Portland, OR). Heating rates were calculated as °C/g, °C/min, °C/g/min. Total and drip cooking losses were calculated for each loin roast. Evaporation loss was calculated as the difference between total cooking loss and drip loss.

Warner-Bratzler shear values. Duplicate samples from each muscle portion were cut across the predominant longitudinal fiber direction (Fig. 1) using a 1.27 cm core meat sample on a Warner-Bratzler shear apparatus (25 kg × 50g dynamometer scale, G.R. Electric Mfg. Co., 1317 Collins Lane, Manhattan, KS).

Table 1—Visual display/outlay of the pork and lamb loin cooking temperature-endpoint temperature combinations, used for this central composite rotatable design with two independent variables: cooking temperature [°C (°F)] and endpoint temperature (°C)[a].

Endpoint temperature (°C)		Cooking temperature, °F (°C)				
		300 (149)	322 (161)	375 (191)	428 (220)	450 (232)
Pork	Lamb					
95.0	90.0			*		
92.4	85.6		*		*	
86.0	75.0	*		* *** *		*
79.6	64.4		*		*	
77.0	60.0			*		

[a] Asterisks represent replication of meat sample for designated cooking and endpoint temperatures.

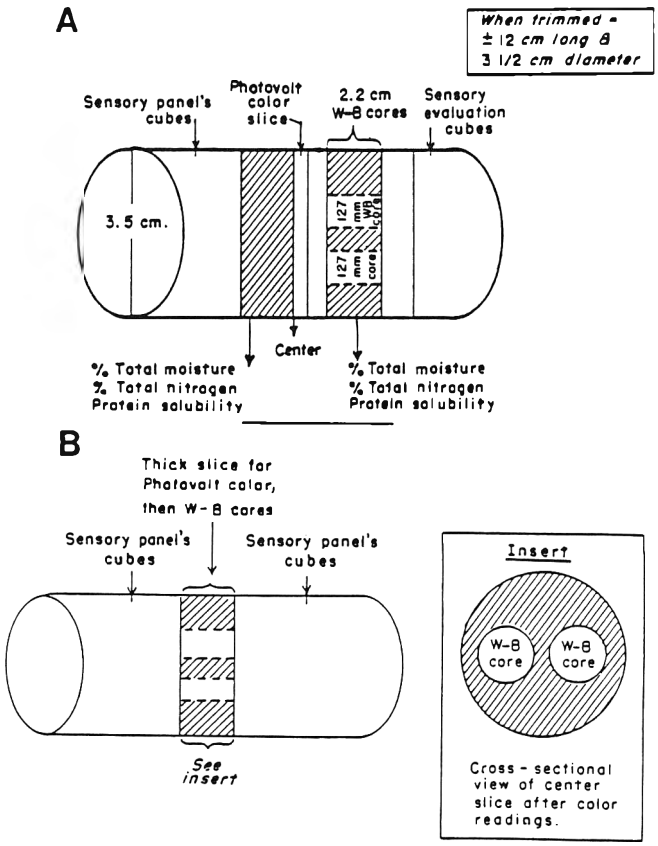

Fig. 1—Sampling diagram of excised longissimus dorsi muscles from pork and lamb loin roasts cooked according to central composite rotatable design and specified cooking temperature and endpoint temperature combinations for chemical and sensory tests (A = pork; B = lamb).

Total moisture and water-holding capacity. Total moisture content was determined on duplicate 5g cooked, chopped longissimus dorsi samples, according to the AOAC vacuum oven method (AOAC, 1980). The method of Wierbicki and Deatherage (1958) was used to determine the water-holding capacity. The expressible moisture index (EMI) values were calculated as the ratio between the mean meat and mean juice areas.

Total nitrogen and protein extractions. Total nitrogen content (μg N/mg meat, wet and dry weight basis) was determined on duplicate samples (Fig. 1) of the finely chopped cooked and raw pork and lamb (micro-Kjeldahl method, AOAC, 1980). The extraction method used for both low ionic strength (LIS) soluble protein extract and nonprotein nitrogen (NPN) extract/fraction was that of Hegarty et al. (1963). Data collected were used for calculating percent total nitrogen, sarcoplasmic fraction (LIS - NPN), remaining protein fraction (TN - (LIS + NPN)) high ionic strength soluble protein fraction(s), alkali-soluble protein and connective tissue residue (Hegarty et al., 1963).

Color measurement. Color of an inner slice (Fig. 1) cooked muscle was determined as percent reflectance (Photovolt Reflectance Meter, Photovolt, New York, NY 10010; amber, 21.0; blue, 25.0; green 23.5). Duplicate amber (A), blue (B) and green (G) filter values were recorded and averaged. CIE chromaticity coordinates x, y and z and saturation indices were calculated (*Gardner's Color Scale Conversion Equations*, 1980).

Sensory tests

Eight and six Oregon State University staff members were selected through preliminary screening and trained to evaluate sensory characteristics for pork and lamb, respectively. They evaluated tenderness (5 = very tender, 1 = very tough), flavor (5 = very pronounced meaty flavor, 1 = no meaty flavor), doneness (5 = very overcooked, 1 = very undercooked), juiciness (5 = very juicy, 1 = very dry), and color. Color descriptions were slightly different for the two species (5 = greyish brown, 1 = rosy pink for the cooked pork and 5 = brownish grey and 1 = rosy red for the lamb meat).

In both cooked pork and lamb, four coded 150 mm cubes (Fig. 1) were presented to each panelist. Color was evaluated on meat slices using light representative of daylight at high noon on a cloudy day (Executive Daylight Lamp BBx-324, Newbursh, New York 12550). The samples were evaluated at room temperature (21°C).

Table 2—Analysis of variance significant variables for fresh pork loin and frozen lamb loin for the two-factor central composite rotatable design (CCRD)

	P-values/Significance values	
Y-variables	Pork[a]	Lamb[a]
Heating rate, °C/g	N.S.[b]	0.08
Heating rate, °C/min,	0.01	0.07
Heating rate, °C/g/min	N.S.	N.S.
Total cooking loss, %	N.S.	<0.0001
Drip loss, %	N.S.	0.0002
Evaporation loss, %	0.08	<0.0001
Cooking time, min	0.04	0.0086
Expressible moisture index	N.S.	0.03
Percent total moisture	0.05	0.07
Warner-Bratzler, kg/1.27 cm	N.S.	N.S.
Total nitrogen, wet wt	N.S.	0.01
Low ionic strength, wet wt	N.S.	N.S.
Non-protein nitrogen, wet wt	N.S.	N.S.
Sarcoplasmic protein fraction, wet wt	N.S.	N.S.
Remaining protein fraction, wet wt	N.S.	N.S.
Total nitrogen, dry wt	0.001	N.S.
Low ionic strength, dry wt	N.S.	N.S.
Non protein nitrogen, dry wt	N.S.	N.S.
Sarcoplasmic protein fraction, dry wt	N.S.	N.S.
Remaining protein fraction, dry wt	0.001	N.S.
Chromaticity coordinate, x	N.S.	0.007
Chromaticity coordinate, y	N.S.	N.S.
Chromaticity coordinate, z	0.039	0.002
Saturation index	N.S.	0.08
Tenderness[c]	N.S.	N.S.
Flavor[c]	N.S.	N.S.
Doneness[c]	N.S.	0.006
Juiciness[c]	0.10	N.S.
Color[c]	N.S.	0.008

[a] Single regressions were done on the cooking loss data, cooking times and heating rates for pork versus double regressions on lamb.

[b] N.S. means "Not Significant"

[c] Sensory evaluation (tenderness: 5 = very tender, 1 = very tough; flavor: 5 = very pronounced meaty flavor; doneness; 5 = very overcooked, 1 = very undercooked; juiciness: 5 = very juicy, 1 = very dry; color: 5 = greyish brown, 1 = rosy pink for cooked pork and 5 = brownish grey and 1 = rosy red for lamb meat).

Statistical data analysis

The following quadratic polynomial regression equation (model) was assumed for evaluating the individual Y-variables:

$$\hat{Y} = \alpha + \beta_1 CT + \beta_2 ET + \beta_{11} CT*CT + B_{22} ET*ET + \beta_{12} CT*ET \quad (1)$$

where, $\hat{Y}$ = the predicted Y-variable's value; CT = cooking temperature, °F; ET = endpoint temperature, °C.

The lamb experiment was greatly strengthened, statistically, by the opportunity to check the quadratic fit to both right and left sides. There were no inconsistencies between sides.

The quadratic models were used to create the 3-dimensional response surfaces (Fuhrer, 1984) for each significant variable. In the response surfaces, CT and ET are located along the traditional X and Y-axes, respectively, while the response variable falls along the Z-axis perpendicular to the X-Y axes. Shaded areas indicate minimum or maximum regions or saddles.

RESULTS & DISCUSSION

TABLE 2 lists pork and lamb meat characteristics with P-values, if significant. For both species total cooking time (min); heating rate (°C/min); evaporation cooking loss (%); total moisture (%); and chromaticity coordinate, z, differed significantly ($P \leq 0.10$). Other dependent variables were shown to be significantly influenced by CT-ET combinations. For pork, the differences were total nitrogen (μg N/mg pork, dry weight basis), remaining protein fraction (μg N/mg pork, dry weight basis), and juiciness as scored by the sensory panel. For lamb, additional significant dependent variables included heating rate (°C/g), total cooking loss (%), drip loss (%), expressible mois-

ture index, total nitrogen (μg N/mg, wet weight basis), chromaticity coordinate x, saturation index, and sensory doneness and color. The nature of the relationship between the independent and dependent variables is seen by the three-dimensional response surfaces.

Tables 3 and 4 give significant, estimated $\hat{Y}$ partial regression coefficients for the quadratic model shown in Eq. (1). These may be used to predict response values for significant Y-variables at other CT-ET (CT is in °F as customary oven temperatures were used and ET is in °C) combinations within the ranges of CT-ETs used in this study. This might be of special interest to the meat industry and/or institutions when wishing to predict cooking losses and cooking times.

Response surfaces

Cooking time. The computer-calculated cooking times for the range of ETs were 74.7 to 242.5 min and from 36.7 to 94.3 min for pork and lamb, respectively. From the pork response surface (Fig. 2A), it will be noted that the longest cooking time (242.5 min) came at the lowest CT 300°F (149°C) and highest ET (95°C) combination as would be expected (Brady and Penfield, 1982). Cooking time was not a linear relationship at CTs below 405°F (207°C). An increase in cooking time occurred in the range 135 to 160 min at CT below 405°F (207°C). The cooking time decreased when the CT was above 405°F (207°C).

The lamb response surface (Fig. 2B) also showed that the longest cooking time came at the lowest CT 300°F (149°C) and highest ET (95°C) combination. The surface of the response surface is slightly curved and is not a linear plane. There was no maximum peak found in this variable. Judging from the number and direction of contour lines, another factor to consider is that ET has more of an effect than does CT. Heating rate (°C/g) (Fig 2E) and cooking time surfaces were similar in shape. (Heating rate (°C/g) was not significant for pork.)

Heating rate, °C/min. The 3-dimensional response surface (Fig. 2C) for heating rate (°C/min) of pork loins shows contour lines increasing in an almost linear fashion. As the CT-ET combination increased along the X-axis (cooking temperature), there was a linear trend toward a heating rate increase; whereas, along the Y-axis (ET) there was only a slight increase at the minimum CT-minimum ET and maximum CT-maximum ET junctions. Since cooking temperature is not a function in the quadratic formula for calculating this variable, heating rate (°C/min) is shown to be a function of ET and not CT. The increased curvature for lamb (Fig. 2D) reveals a decreasing rate from about 63°C to 90°C at lower CTs, but with CTs above 375°F (191°C), there was a rapid heating rate increase across all ETs. Between 75 and 79°C ET there is a valley floor. This and the values for heating rate, °C/min, may relate to the endothermic protein denaturation reactions or moisture evaporation (Cloke et al., 1981). The differences between the heating response surfaces in pork and lamb could be partially reflective of frozen versus fresh samples; however, of greater importance could be sample size. Pork roasts were 40% heavier.

Cooking losses. Plots for total and drip cooking loss for lamb loin roasts only are shown in Fig 3, A and B, as the effects for pork roasts were not significant at $P \leq 0.10$. Although total cooking loss (%) is a function of CT, ET has more influence. The response surfaces show the highest cooking losses at the highest ETs, especially above 75°C. As expected, maximum loss occurred at the maximum CT-maximum ET region. The highest loss (stationary point) was at 31.96%. This indicates that this combination of CT-ETs came close to the maximum value, as there is a definite hill present in the maximum CT-maximum ET "corner." Therefore, in roasting lamb, it would be best to avoid higher ET ranges (at any CT) especially 75°C ET and above, in order to obtain better yields.

In analyzing pork and lamb data, evaporation loss (%) was the only common significant cooking loss variable. The pork response surface (Fig. 3C) shows a ridge cresting at about 375°F (191°C), increasing steadily from minimum to maximum ET. A high degree of evaporation took place between about 350° and 400°F (177° and 204°C), especially at the maximum endpoint temperature (ET) of 96°C. The amount of evaporation shown above 85°C was much higher than at the minimum CT-minimum ET and maximum CT-maximum ET regions. A CT between 350° and 400°F (177° and 204°C) resulted in the highest amount of evaporation loss in pork loin roasts. This loss increased from low (75°C) to high (95°C) endpoint temperatures.

For lamb, evaporation loss (%) response surface (Fig. 3D) resembles the other two lamb cooking loss surfaces, total (Fig. 3A) and drip (Fig. 3B). As with the pork, the lamb response surface (Fig. 3D) shows increasing evaporation loss (%) values from minimum to maximum ET. A high degree of evaporation took place between about 375° and 450°F (191° and 232°C), and, as expected, this occurred mainly around the maximum endpoint temperatures (ETs) of 87–90°C. Thus, the lower CT and ET ranges are preferable for reducing evaporation loss in both lamb and pork loin roasts.

The significant lamb drip loss (%) surface (Fig. 3B) also indicates a high (11.45%) in the region of high CT-ET combinations. This drip was primarily fat and extractives (Lind et al., 1971). The surface (Fig. 3B) is decidedly curved, especially in comparison to total cooking (Fig. 3A) and evaporation (Fig. 3D) losses. The maximum ET-minimum CT area is lower in this case than the response surface of total cooking loss (%). The results shown in Fig. 3 are supported in work reported by a number of other researchers. Studies concerning the internal temperature of pork (Bowers and Goertz, 1966; Webb et al., 1961) have shown that cooking loss increased as internal temperature of pork roasts increased. Brady and Penfield (1982) found that hot water bath and oven-roasted samples heated to

Table 3—Quadratic regression model coefficients for pork loin roast for substitution into Eq. (1) (with cooking temperature in °F and endpoint temperature in °C)[a,b]

	A (constant)	CT × 10⁻²	ET × 10⁻²	CT² × 10⁻⁶	ET² × 10⁻³	CT*ET × 10⁻³
Cooking time, min	−907.9	88.61	2222.0	0	−23.16	−45.42
Heating rate, °C/min	3.947	−0.5929	−7.005	0.2778	0.2142	0.1029
Cooking loss						
Total (%)	−357.3	124.8	302.5	−792.8	0.5741	−7.244
Evaporation (%)	−98.62	66.86	−62.29	−727.7	9.413	−1.33
Expressible moisture index	−2.543	0.0869	6.163	4.881	−0.2219	−0.05717
Total moisture, %	494.2	−84.7	−61.11	16.26	12.63	9.756
Total nitrogen, μg N/mg meat dry wt basis	209.1	−329.1	−310.1	51.99	10.06	3.455
"Remaining" protein μg N/mg meat	1.795	−299.8	−2.605	0.3032	−0.7623	0.03326
Chromaticity coordinate, z	−0.4416	−.1109	2.283	1.117	−0.1421	0.003486
Juiciness sensory score means[c]	63.89	4.173	−157.4	−72.72	8.807	0.1148

[a] $\hat{Y} = \alpha + \beta_1 CT + \beta_2 ET + \beta_{11} CT*CT + \beta_{22} ET*ET + \beta_{12} CT*ET$

[b] Cooking temperature (°F), endpoint temperature (°C)

[c] Juiciness: 5 = very juicy, 1 = very dry

Table 4—Quadratic regression model coefficients for lamb loin roast for substitution into Eq. (1)[a,b]

	A (constant)	CT × 10^{-2}	ET × 10^{-2}	CT^2 × 10^{-6}	ET^2 × 10^{-3}	CT*ET10^{-4}
Cooking time, min	−132.8	−34.68	659.3	72.12	−24.68	−47.09
Heating rate, °C/g	0.1288	−0.04988	0.1253	−0.4943	−0.1464	0.1036
Heating rate, °C/min	4.505	0.3527	−11.51	−3.667	0.6467	0.3056
Cooking loss						
Total (%)	−226.200	61.42	26.74	−755.1	−13.80	−1.212
Drip (%)	−111.4	27.05	147.4	−44.72	−10.64	11.03
Evaporation (%)	−114.8	34.37	120.0	−30.79	−3.164	−9.819
Expressible moisture index	−0.1256	0.07076	0.2978	−4.429	−0.09016	0.3867
Total moisture, %	70.82	−4.453	37.59	13.77	−1.952	−8.225
Total nitrogen, μg N/mg meat wet wt basis	0.05340	59.37	−0.3621	−0.699	0.02838	−0.0083
Cooking time, min	−132.8	−34.68	659.3	72.12	−24.68	−47.09
Chromaticity coordinate, x	1.400	−0.202	−1.644	1.964	0.08504	0.06507
Chromaticity coordinate, y	−0.2915	0.38	0.7991	−0.2762	−0.4595	−0.02447
Chromaticity coordinate, z	−0.3815	0.167	0.8613	−1.733	−0.4028	−0.04055
Saturation index	175.0	−25.37	−28.75	192.8	14.44	12.77
Doneness[c]	−7.652	−0.067	24.37	−18.15	−1.83	1.902
Color[d]	−49.82	12.72	69.47	−19.12	−4.928	2.96

[a] $\hat{Y} = \alpha + \beta_1 CT + \beta_2 ET + \beta_{11} CT*CT + \beta_{22} ET*ET + \beta_{12} CT*ET$

[b] Cooking temperature (°F), endpoint temperature (°C).

[c] Doneness: 5 = very overcooked, 1 = very undercooked

[d] Color: 5 = brownish grey, 1 = rosy red

70°C lost more moisture than those heated to 60°C, and the samples heated at a faster rate lost less moisture than those at a slower rate.

All three of the cooking loss (%) measurements for lamb as plotted, show that the CT-ET combination is approaching a stationary maximum point within a maximum region (Fig. 3A, C and E).

Percent total moisture. The pork response surface (Fig. 4A) for total moisture shows a definite minimax-saddle arrangement, indicating that one of the X-variables (CT or ET) is at its maximum while the other is at its minimum. There is an "iso-moisture" content area which falls between 60.7 and 61.8% total moisture. This phenomenon occurred roughly between 85° and 87°C ET for low CTs and 83° and 86°C for higher CTs, and between 415°F (213°C) and 425°F (218°C) CT for the low ETs and between 380°F (193°C) and 395°F (202°C) for the higher ETs. A more detailed evaluation of the surface reveals that along 300°F (149°C) CT at the lower ETs, there is more total moisture retained. This is clearly noticeable as a "pointed corner" in the response surface. The opposite corner also peaks and the valley or plateau region is also visible in this surface viewpoint. The peaked area in the high CT-high ET region ties together with the short cooking time and low evaporation losses in this region. Response surface analysis (RSA) permits the recommendation of a CT between 400-450°F (204–232°C) and an endpoint temperature between 87° and 95°C, in order to obtain a moist, well-cooked roast. The total moisture (%TM) data show that the quadratic model and the response surface are useful in explaining what is happening in terms of the response of the dependent variable to the effect of the two independent "heat" variables.

For both pork and lamb, the longer the cooking time, the lower the %TM, with %TM leveling off in an iso-moisture region. The valley changes direction, possibly due to the change in the water-holding capacity of the muscle proteins (Hamm, 1966). The protein peptide chains unfold at internal temperatures below 65°C and form unstable cross-linkages followed by partial denaturation of the sarcoplasmic proteins. The myofibrillar proteins become tougher and there is a loss in WHC. Collagen shrinks between 61–63°C and softens as the secondary (helical) structure of the protein is destroyed. Above 65°C final coagulation of the myofibrillar and sarcoplasmic proteins occurs, with corresponding explusion of water. Due to the temperature gradient phenomenon (Cloke et al., 1981), not all the proteins undergo denaturation and coagulation simultaneously. This could be partly responsible for the iso-moisture region.

Expressible moisture index. On the lamb response surface (Fig. 4C), the highest EMI values are found in the maximum

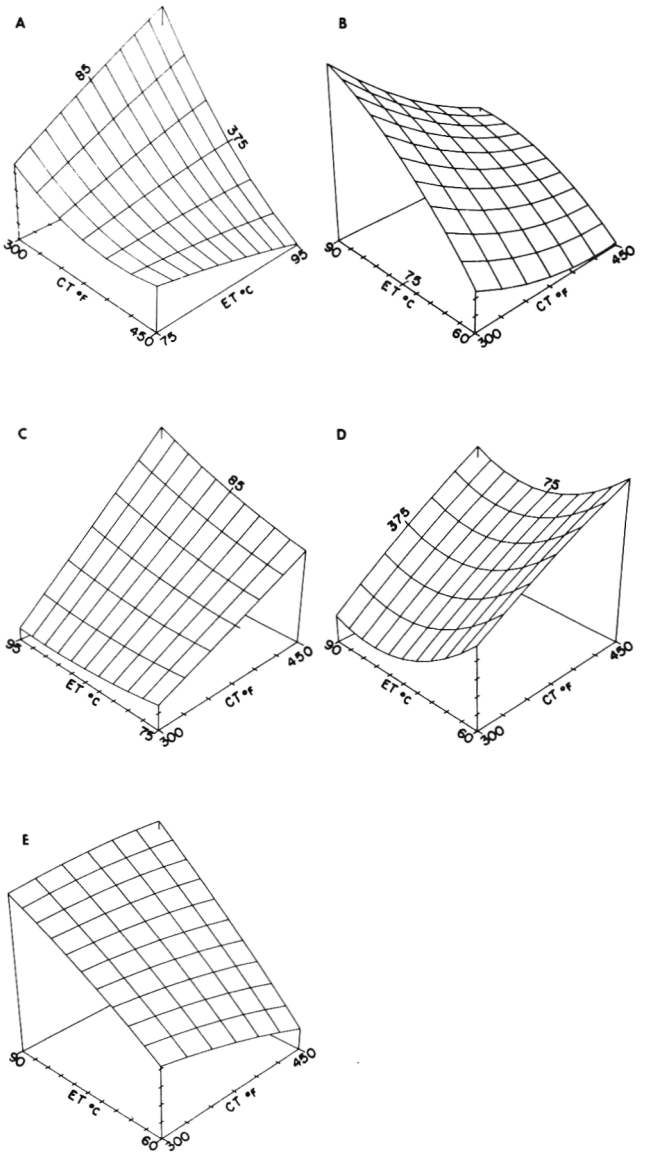

Fig. 2—Pork and lamb loin roasts response surfaces with independent variables of cooking (oven) temperature (CT, x-axis), °F, and endpoint temperature (ET, y-axis), °C for the response variables (z-axis): total cooking time (min), pork (A) and lamb (B); heating rate (°C/min), pork (C) and lamb (D); and heating rate (°C/g), lamb (E).

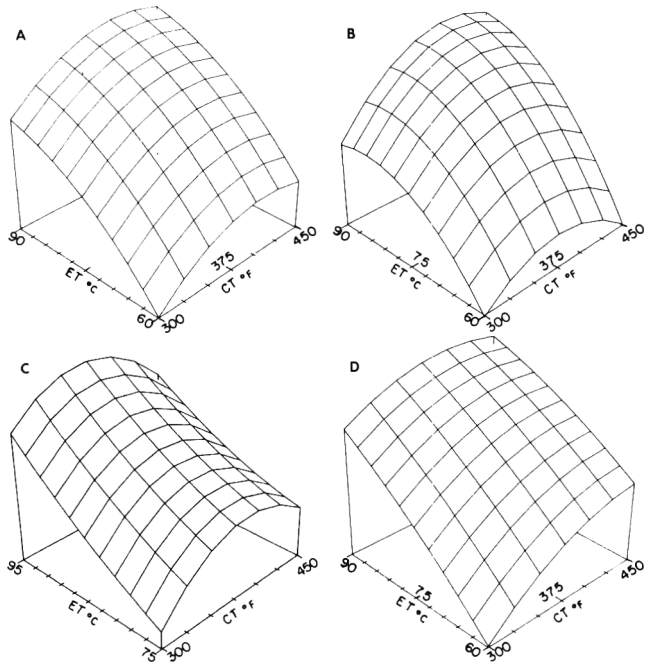

Fig. 3—Lamb and pork loin roasts response surfaces with independent variables of cooking (oven) temperature (CT, x-axis), °F, and endpoint temperature (ET, y-axis), °C for the response variables (z-axis): cooking loss (%), lamb (A); drip loss (%), lamb (B); evaporation loss (%), pork (C) and lamb (D).

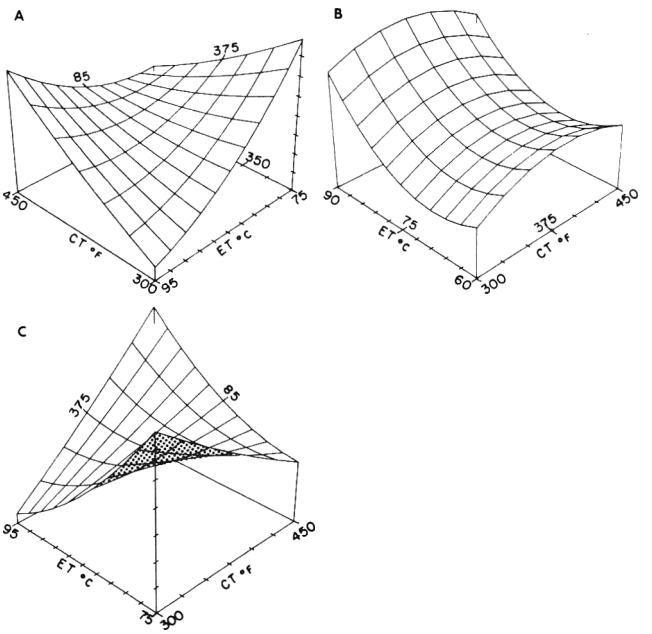

Fig. 5—Pork and lamb loin roasts response surfaces with independent variables of cooking (oven) temperature (CT, x-axis), °F, and endpoint temperature (ET, y-axis), °C for the response variables (z-axis): total nitrogen (μg N/mg meat), pork (dry weight basis, A) and lamb (wet weight basis, B); and remaining protein (μg N/mg meat), pork (dry weight basis, C).

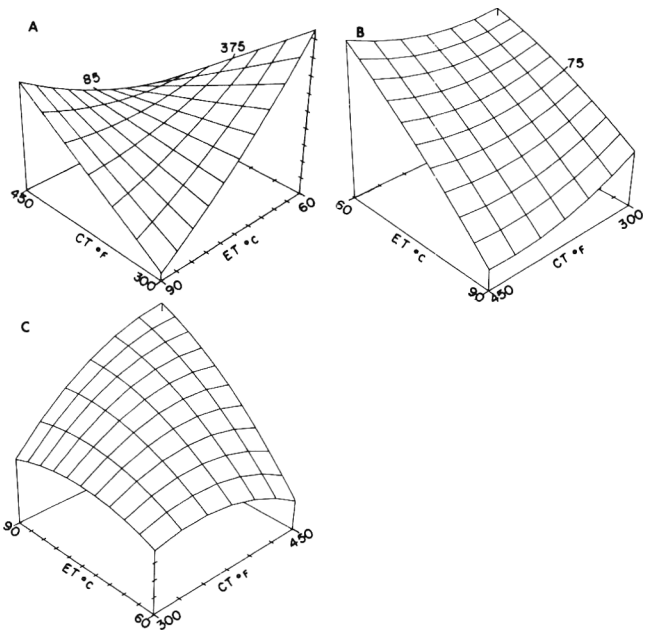

Fig. 4—Pork and lamb loin roasts response surfaces with independent variables of cooking (oven) temperature (CT, x-axis), °F, and endpoint temperature (ET, y-axis), °C for the response variables (z-axis): total moisture (%), pork (A) and lamb (B); and expressible moisture index, lamb (C).

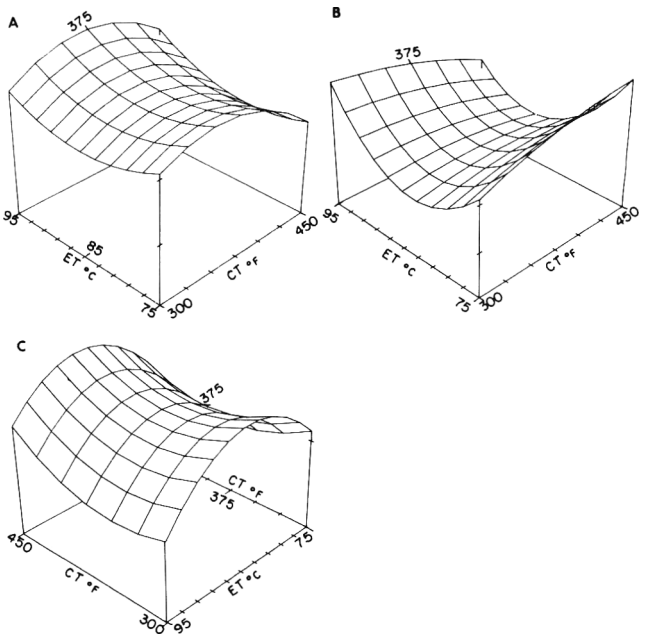

Fig. 6—Pork loin roast response surfaces with independent variables of cooking (oven) temperature (CT, x-axis), °F, and endpoint temperature (ET, y-axis), °C for the C. I. E. chromaticity coordinate response variables: x (A); y (B); and z (C).

CT-maximum ET area where the contours are approaching a maximum region indicating less WHC here. Pork EMI values were not significantly affected by the CT-ET combinations used.

Total nitrogen and protein solubility. For pork, only the amount of total nitrogen (TN, μg N/mg pork, dry weight basis) and the amount of remaining protein (REM), dry weight basis, are significant ($P = 0.001$). The pork contour plot (Fig. 5A) shows that the amount of total nitrogen retained decreased as one moves from 300°F (149°C) towards 450°F (232°C) CT, on the 75°C ET line. For a given CT, for example, 325°F (163°C), as one increases ETs, the amount of total nitrogen retained decreases. Around 85°C ET and between 400–425°F (204–218°C) CT, TN remains fairly stable as represented by the saddle. The lowest values for TN occur at the min CT-max ET corner with the best retention in the min CT-min ET corner. This resembles the %TM (Fig. 4A) very closely. The water-holding capacity of the proteins is altered through den-

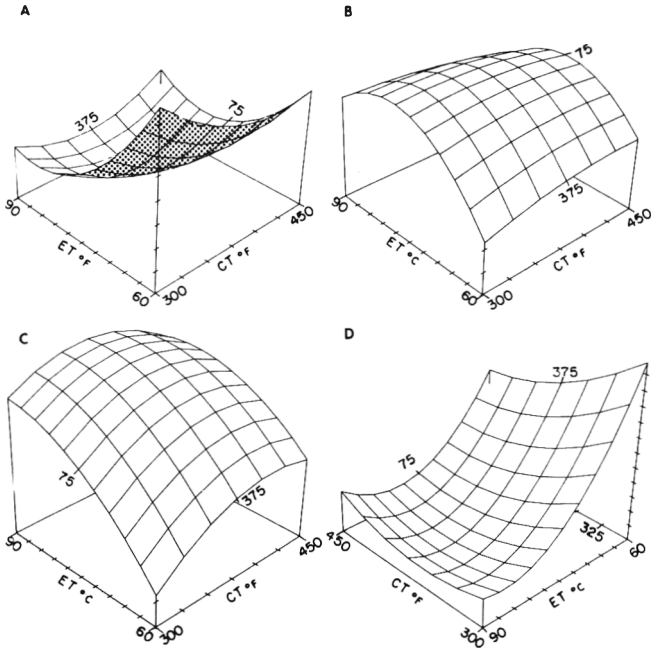

Fig. 7—Lamb loin roast response surfaces with independent variables of cooking (oven) temperature (CT, x-axis), °F, and endpoint temperature (ET, y-axis), °C for the C. I. E. chromaticity coordinate response variables: x (A); y (B); and z (C); and for saturation index (D).

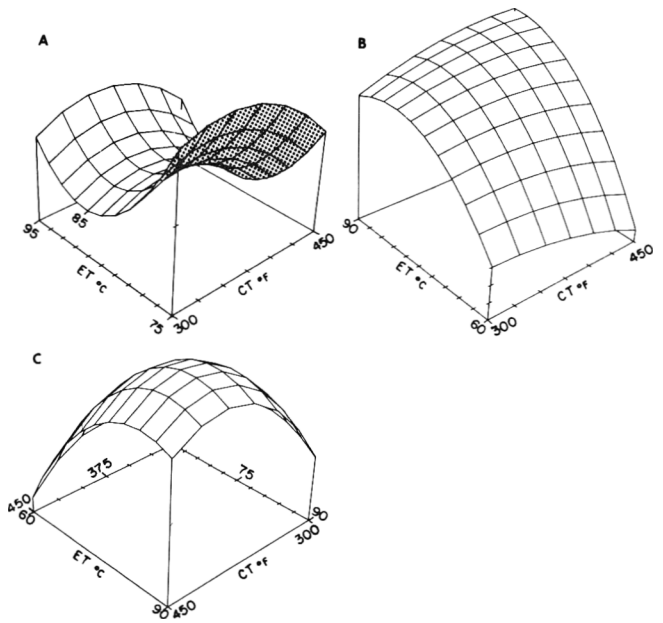

Fig. 8—Pork and lamb loin roast response surfaces with independent variables of cooking (oven) temperature (CT, x-axis), °F, and endpoint temperature (ET, y-axis), °C for the sensory response variables (z-axis): juiciness, pork (A); doneness, lamb (B); and color, lamb (C).

aturation and coagulation, and the amount of moisture held varies in a proportional way with the amount of total nitrogen retained. This could suggest that in the moist milieu of proteins (low CT-low ET and high CT-high ET) there is less severe damage to the tertiary and quaternary structure of the protein backbones/chains. The response surfaces of the remaining protein fractions (dry weight) (Fig. 5C) for pork for almost identical to the TN ones, although the values are slightly different.

In lamb (Fig. 5B), only wet weight basis values for total nitrogen differed significantly. This relates to the amount of moisture present in the samples, as discussed previously. The quadratic regression model and 3-dimensional surfaces explain the response of total nitrogen on a wet weight basis to the significant effect of CT and ET. The saddle visible in Fig. 5B shows an area where the values are almost constant at a wide array of temperatures. The least amount of lamb total nitrogen retained remained almost at a constant low in the valley running between 66° and 75°C across the whole CT range, moving from 300°F (149°C) towards 450°F (232°C) CT. For a given CT, for example 375°F (191°C), as ETs increase the amount of total nitrogen retained at first slightly decreased and then increased again. The least amount of protein over-coagulation would be expected in the low CT-low ET and in the high CT-high ET regions.

Color. In pork (Fig. 6), the CIE chromaticity coordinate z (Fig. 6C) was significant ($P=0.04$), whereas x and y were not. It is necessary to consider all these values for the CIE chromaticity diagram. The response surface of the pork coordinate z (Fig. 6C) shows a saddle; however, it is the least informative of the "responses" being considered. In lamb (Fig. 7) the chromaticity coordinate x (Fig. 7A) and z (Fig. 7C) values were significant. The three CIE chromaticity coordinates (Fig. 7A, 7B, and 7C) of the lamb color measurements show that maximum areas have been encountered. Coordinates x, y, and z show peak response values. When using the peak values for plotting the x and y coordinates, the computed maximum x, y and z values are 0.345, 0.349, and 0.313, respectively. They are located in the CIE Illuminant C area, towards the purplish-pink region, for both lamb and pork (DeMan, 1980). The saturation index information (Fig. 7D) shows a slight bowl-valley configuration, with the minimum region clearly shown. The valley falls slightly above the center point CT-ET area, across the CT range, from about 78°C ET and upwards to 90°C.

Sensory evaluation. Juiciness (Fig. 8A) was the only pork sensory quality characteristic significantly ($P=0.10$) affected by CT-ET combinations. This is in agreement with the % total moisture and % total cooking loss information referred to previously. The response surfaces emphasize a definite valley in the region of 2.5–3. When the ET was low (below 80°C) or high (above 90°C) (shaded area), the juiciness values were high (above 3); whereas, the lowest juiciness values (below 2.5) were found at the highest CT 450°F (232°C) at virtually any selected ET. There is a wide saddle area around the maximum range covering the 80–84°C ET range and the total CT range.

Two related lamb sensory quality characteristic variables were significant, namely doneness ($P=0.006$) (Fig. 8B) and color ($P=0.008$) (Fig. 8C). The doneness response surface (Fig. 8B) shows the lamb cooked to higher ETs (79°C and above) was judged to be more well-done, especially with higher CTs of 375°F (191°C) and above. The relationship between the lower CTs and ETs and the higher ones is almost linear, with the higher values from approximately 75°C and above. The peak is almost attained, with the stationary point at approximately 3.2, a value near the "neither-overcooked nor undercooked" score value of 3. Judging from the surface (Fig. 8B), the ET is again more important than the CT, as more lines are crossed when one moves upwards vertically from the 60°C ET line.

The lamb sensory color minimum and maximum values are 0.4 and 4.0, respectively. The peak area is at 4.0 (slightly overcooked), and this peak is obvious in the response surface (Fig. 8C). This is almost in the same area as for doneness (Fig. 8B). The lowest scores are in the minimum CT-minimum ET corner, or the most reddish-pink area, and are typical of undercooked meat.

The integration of the central composite rotatable design and response surface analysis enhanced the interpretation of the influence and interrelationships of cooking temperature and endpoint temperature on pork and lamb quality characteristics.

The results of varying cooking temperatures and/or endpoint temperatures in this experiment generally conform to those expected from the literature. However, the data analysis explained anomalies which have been reported. It is evident from the curved and/or saddle-shaped surfaces, particularly for lamb drip loss and pork total moisture and total and remaining nitrogen (dry weight basis), that CT or ET selection may bias the determination of the differences and relationships to quality characteristics.

The information gleaned from response surfaces of both significant and nonsignificant variables may be put to practical use in industry, by institutions and the consumer alike. The surfaces themselves are valuable resources as they can be used for estimating the range of response values relevant for a particular CT-ET combination of interest for a variable. For example, the nature of the pork juiciness plots (Fig. 8A) shows that the trained panel could not distinguish between the effects of a range of CT-ET combinations. Thus, other important factors may be used for decision-making such as fuel saving measures and economy factors.

This central composite rotatable design (CCRD) may be used for predicting the behavior of selected dependent variables of roast pork and lamb loins, but also, perhaps more importantly, for examining the effect of the CT-ET variables and using the contour plots and response surfaces to aid decision-makers, providing the temperature combinations being evaluated are within those tested.

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($P<0.05$) among the textural parameters measured, with the exception of no significant variation in springiness. The KC treatment was the most resistance to compression force of the low-fat treatments, in contrast to IC which was the least resistant. Both carrageenan treatments were not significantly different from CW but IC and CW were significantly different ($P<0.05$) from CF. A decrease in cooked batter firmness due to an increase in added water was also observed by Johnson et al. (1977). There was no significant correlation ($P>0.05$) among sensory and texture values, as expected, because texture was only one of the many parameters evaluated in deciding a hedonic score. The distribution of hedonic scores is seen in Fig. 5. Data show that for all treatments the majority of scores lie above the neutral value (5), indicating that panelists scored more frequently on the "like" than the "dislike" side of the scale.

CONCLUSIONS

OF THE GUMS EVALUATED in this study, kappa and iota carrageenan appear to be the most beneficial in manufacturing low-fat frankfurters. Both carrageenans appeared to help hold moisture in the cooked product and kappa carrageenan increased the hardness. However, sensory evaluation indicated that the significant variation in texture did not have a significant influence on hedonic scores and that all low-fat treatments were as acceptable as control frankfurters.

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A Comparison of Some Properties of Meat from Young Buffalo (*Bubalus bubalis*) and Cattle

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ABSTRACT

Meat properties of four muscles of differing collagen content from both Achilles tendon-hung and tenderstretched (suspended from the sacrosciatic ligament) sides of water buffalo (*Bubalus bubalis*) and Brahman-cross steers were assessed. The animals were together on good pasture until slaughtered at similar ages (27 months) and liveweights (ca 317 kg). Buffalo muscles were darker than beef and darkened more at chill temperatures. Warner-Bratzler peak force minus initial yield values, adhesion and compression values indicated that for the three muscles with the greater connective tissue contents, buffalo muscles were slightly tougher than corresponding beef muscles because of a greater contribution of connective tissue to toughness. No species differences were found for the psoas major muscle except for flavor, where beef was slightly preferred.

INTRODUCTION

IN A PREVIOUS EXPERIMENT (Robertson et al., 1983), a number of objective and subjective assessments were made of the quality of meat from water buffalo (*Bubalus bubalis*) and beef (Brahman-cross) cattle aged 51 months. The results for these older animals showed that buffalo muscles were darker than the equivalent beef muscles even though their ultimate pH values were not significantly different. They also showed that, while differences in mechanical strength of the cooked muscles were not large, they were statistically significant. Most of the evidence indicated that the connective tissue in the buffalo meat had a bigger contribution to toughness than it did in beef.

Ragub et al. (1966) considered that meat from Egyptian buffalo was unacceptably tough when animals were older than 24 months. Our previous experience indicated that this is not necessarily so, at least for some muscles of Australian buffalo. Longissimus dorsi (LD) muscles from pelvic-hung sides of 51 month-old buffalo were found by our taste panel to be acceptable for tenderness. Matsukawa et al. (1976) found that LD muscles from young buffalo, 19–25 months of age, had similar mean Warner-Bratzler (WB) peak shear force values and taste panel scores to those from Sinhala, Red Sindhi and Friesian cattle of similar age, 19.5–21 months.

Following the earlier work of Robertson et al. (1983), it was decided to determine whether similar differences existed between buffalo meat and beef from younger animals.

MATERIALS & METHODS

Animals and animal treatment

Nine buffalo steers were weaned at 11–15 months of age (mean, 13 months) at the Coastal Plains Research Station, N.T. Approximately 8 months later and 6 months before eventual slaughter, nine Brahman-cross steers, matched with the buffalo on an age and live weight basis, were brought from Tortilla Flats Research Farm, N.T., to the Coastal Plains Research Station. The buffalo and Brahman-cross steers were then grazed there together on improved or native pasture until all were slaughtered at an abattoir 30 km away. The animals were then 24–30 months old (mean, 27 months).

Slaughter and postmortem procedures

The animals were weighed full, fasted for 24 hr and reweighed. They were then transported to the abattoir where they were held overnight in pens with water available and slaughtered between 38 and 42 hr after the first weighing.

After dressing, the carcasses were split. One side was hung by the Achilles tendon while the other side was rehung from the sacrosciatic ligament (tenderstretched) in the cooler within 1 hr of slaughter. The sides were chilled for 48 hr at 0–2°C. Semitendinosus (ST), longissimus dorsi (LD), semimembranosus (SM), and psoas major (PM) muscles were then removed from each side, trimmed of extraneous fat, placed in polyethylene bags and frozen in cartons at −20°C. After a week at −20°C, the cartons were transported by refrigerated truck from Darwin to the CSIRO Meat Research Laboratory where they were stored at −32°C until required. All muscles except the PM were thawed on wire racks in a chiller at 0–1°C for 48 hr; the PM muscles were thawed at 5–6°C for 24 hr.

Muscle treatment and cooking methods

After thawing, a 2–3 cm long portion was removed from both ends of each ST muscle. The remaining portion was cut transversely into three parts measuring about 8, 5, and 8 cm. The mid-section (5 cm long) was used for color measurements. The other two sections (8 cm) were trimmed of obvious connective tissue to give two samples measuring about 8 × 5 × 5 cm, each weighing about 200g. Each sample was then cooked at 80°C for 90 min and used to provide samples for WB shear and adhesion force measurements.

Each thawed SM muscle had a transverse slice 5–7 cm thick removed from the proximal end for color measurements and a 6–8 cm transverse slice from the distal end for pH measurements. A longitudinal slice from the anterior side of the muscle was removed for sarcomere length and other measurements. Samples weighing about 150g were cooked at 60° or 80°C for 1 hr for WB shear measurements.

After thawing, each PM muscle was trimmed of obvious external fat and connective tissue and divided transversely into three parts. One of these was assigned for taste panel assessment, another for objective measurement and the third for pH and sarcomere length measurement. The PM samples for objective measurement weighed about 160g and were cooked at 80°C for 1 hr. The PM muscle samples for taste panel assessment were sliced transversely into 25 mm thick steaks and cooked by oven grilling on open racks in a forced convection oven at 230°C for 20 min (rotated after 10 min).

The thawed LD muscles were trimmed of all remaining extraneous fat and connective tissue. A sample weighing about 450g was cooked at 80°C for 1.5 hr. After cooking the samples were stored overnight at 0–1°C and divided into two parts. One part provided samples for the taste panel while the other was used for Instron compression and WB shear force measurements.

After the muscles had been thawed and cut into appropriate sizes the samples were dried with paper towels and weighed. They were wrapped in polyethylene bags and cooked, totally immersed, in water baths at the appropriate temperature for the selected time. The cooked samples were cooled in cold running water for 30 min, dried with paper towels and weighed. Each sample was then placed in a polyethylene bag and stored overnight at 0–1°C. Cooking losses were determined from the weights before and after cooking.

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Table 1—Mean Warner-Bratzler shear force, adhesion, sarcomere length and cooking loss for semitendinosus muscles (ST) from buffalo and beef (Brahman-cross) carcasses

	Species/Hanging treatment				LSD[b]	
	Buffalo		Beef			
Parameter measured	AT	TS	AT	TS	A	B
WB peak shear force (kg)	7.62	7.89	6.80	6.52	0.91	0.41
WB initial yield force (kg)	4.15	4.66	5.02	4.78	0.90	0.38
WB peak minus initial yield (kg)	3.47	3.23	1.78	1.74	0.69	0.23
Adhesion (kg)	0.89	0.95	0.84	0.86	0.14	0.08
Cooking losses (%)	36.8	36.6	35.6	33.5	3.0	0.9
Sarcomere length of raw meat (μm)	2.31	2.73	2.19	2.80	0.20	0.08

[a] One side of each carcass was hung from the Achilles tendon (AT) and the other side from the sarcrosciatic ligament (TS).
[b] Least significant difference at P = 0.05 for between species within hanging treatments (A) and between hanging treatments within species (B).

Table 2—Mean Warner-Bratzler shear force and other parameters measured for longissimus dorsi (LD) muscle samples, cooked at 80°C for 90 min, from buffalo and beef (Braham-cross) carcasses

	Species/Hanging method				LSD[b]	
	Buffalo		Beef			
Parameter measured	AT	TS	AT	TS	A	B
WB peak shear force (kg)	10.95	6.18	8.67	4.91	1.95	1.86
WB initial yield force (kg)	8.98	5.18	7.89	4.53	1.96	1.91
WB peak minus initial yield (kg)	1.97	1.00	0.78	0.38	0.69	0.56
Instron compression (kg)	2.85	2.86	2.14	2.14	0.34	0.28
Cooking losses (%)	40.8	36.5	36.8	32.6	1.9	1.0
Sarcomere length for raw meat (μm)	1.62	2.46	1.71	2.86	0.18	0.18
Ultimate pH of raw meat	5.48	5.51	5.54	5.55	0.11	0.10

[a] One side of each carcass was hung from the sacrosciatic ligament (TS) within 1 hr of slaughter, and the other from the Achilles tendon (AT) for 48 hr at 0°C.
[b] Least significant difference at P = 0.05 for between species within hanging treatments (A) and between hanging treatments within species (B).

Table 3—Mean taste panel scores for the tenderness and juiciness of cooked samples (80°C for 90 min) from longissimus dorsi (LD) muscles from buffalo and beef (Brahman-cross) carcasses

	Species/Hanging method				LSD[b]	
	Buffalo		Beef			
Taste panel parameter	AT	TS	AT	TS	A	B
Tenderness[c]	18.70	12.67	15.35	8.74	2.81	1.81
Juiciness[c]	15.48	11.65	14.71	10.82	1.80	1.72

[a] One side of each carcass was hung from the Achilles tendon (AT) and the other from the sacrosciatic ligament within 1 hr of slaughter (TS) for 48 hr at 0°C.
[b] Least significant difference at P = 0.05 for between species within hanging treatments (A) and between hanging treatments within species (B).
[c] Taste panel scales 1 = extremely tender/juicy to 25 = extremely tough/dry.

Sarcomere length and pH measurements

A light diffraction method (Bouton et al., 1973) was used to measure the sarcomere lengths of samples of raw ST, SM, LD and PM muscles. Ultimate pH values were measured directly on samples of the raw muscles at room temperature (ca 22°C) using a Watson Victor 5004 pH meter with a Philips (C64/1) probe type combined electrode.

Objective measurements

The WB shear, Instron compression and adhesion methods have all been described in detail elsewhere (Bouton and Harris, 1972).

Subjective measurements

Tenderness/Juiciness. After overnight storage at 0–1°C the cooked LD muscle samples were cut into 13 mm cubes with meat fiber direction parallel to one side of the cube. The 12 trained (Bouton et al., 1975) panel members were asked to assess four samples for tenderness and juiciness with two cubes per taster per treatment. Samples from a buffalo and from a Brahman-cross were compared at each session, using samples from the sides hung from the Achilles tendon and sacrosciatic ligament, to make a total of four treatments.

Unstructured scales with only the ends defined (extremely tender 1 to extremely tough 25; extremely juicy 1 to extremely dry 25) were used. The panelists marked their score on computer cards. The order of tasting was randomised. Tasting was carried out under green lights to mask color differences.

Flavor assessment. The grilled PM steaks were cut into 19 mm cubes immediately after cooking and kept warm in insulated containers until served to the taste panel; center temperatures of cubes at tasting were 38–40°C. The members of this panel were all experienced and selected (Park et al., 1972) for their ability to detect flavor differences. These panelists were asked to rate four samples (normal and tenderstretched samples from a buffalo plus normal and tenderstretched samples from one of the beef animals) for flavor, juiciness, tenderness, and acceptability. Intensities were rated on 9 point, unstructured, hedonic scales (juiciness: very juicy 0 - very dry 9; flavor, tenderness, and acceptability: very good 0 - very poor 9). Animal numbers were selected randomly and order of sample assessment was randomised. Testing was carried out under green light to mask any color differences.

Meat color measurements. The samples of ST and SM muscles from both sides of each carcass were cut perpendicular to the long axis of their muscle fibers to yield samples of at least 76 mm diameter and 30 mm thick. The freshly cut surfaces were exposed to air, covered with a polyethylene sheet which did not touch the exposed meat surfaces, for 1 hr to allow myoglobin oxygenation.

Meat color was measured according to the manufacturer's instructions, using a Hunter Colorimeter (Model D25M-2, Hunter Associ-

Table 4—Mean Warner-Bratzler shear force, sarcomere length, and cooking loss results obtained from psoas major (PM) muscles from Achilles tendon (AT) and sacrosciatic ligament (TS) hung sides of buffalo and beef (cooked at 80°C for 1 hr)

	Species/Hanging treatment				LSD[a]	
	Buffalo		Beef			
Parameter	AT	TS	AT	TS	A	B
WB peak shear force (kg)	4.28	4.06	4.18	4.68	0.50	0.47
WB initial yield force (kg)	4.04	3.38	3.99	4.22	0.52	0.49
WB peak minus initial yield (kg)	0.24	0.67	0.19	0.47	0.24	0.22
Sarcomere length (μm)	3.28	2.16	3.33	2.21	0.15	0.12
Cooking losses (%)	29.2	31.9	29.1	32.5	1.3	0.8

[a] Least significant difference at P = 0.05 for between species within hanging treatments (A) and between hanging treatments within species (B).

Table 5—Mean taste panel scores for flavor, juiciness, tenderness and acceptability of psoas major (PM) muscle samples from buffalo and beef (Brahman-cross) carcasses

Taste panel parameter	Species & Hanging method				LSD[b]	
	Buffalo		Beef			
	AT	TS	AT	TS	A	B
Flavor[c]	3.44	4.04	2.79	3.10	0.36	0.33
Tenderness[c]	3.07	3.10	2.70	3.23	0.61	0.42
Juiciness[d]	4.20	4.66	3.64	3.71	0.83	0.60
Acceptability	3.77	4.22	3.05	3.48	0.48	0.33

[a] One side of each carcass was hung from Achilles tendon (AT) and the other from the sacrosciatic ligament (TS) within 1 hr of slaughter and carcasses chilled for 48 hr at 0–1°C.

[b] Least significant difference at P=0.05 for between species within hanging treatments (A) and between hanging treatments within species (B).

[c] 0–9, very good to very bad.

[d] 0–9, very juicy to very dry.

ates, VA) with a 50.8 mm diameter specimen port. Care was taken to exclude large areas of intramuscular fat. The C.I.E. Tristimulus (Y, X and Z) and Hunter Opponent colors scale values (L, a and b) were recorded. The meat samples were again covered with a polyethylene sheet and stored at 0–1°C for 24 hr. After this the samples were allowed to warm to room temperature and meat color was measured again.

Statistical methods

Analysis of variance was used to test for significance of treatment effects. Least significant difference (LSD) values were used to determine significant (P = 0.05) differences between treatment means.

RESULTS & DISCUSSION

Liveweight, carcass weight, dressing percentage and fat depth

Neither mean full liveweights (318.4 vs 315.7 kg), mean fasted liveweights (296.0 vs 291.9 kg), mean hot carcass weights (155.9 vs 145.1 kg) nor mean fat depths over the loin (2.4 vs 1.8 mm) differed significantly between the Brahman-cross and buffalo, respectively. Mean dressing percentage was significantly greater ($P<0.01$) for the Brahman crossbreds whether this was calculated on a full (48.9 vs 45.9%) or fasted (52.6 vs 49.6%) liveweight basis. The correlations between fat depth over the loin and hot carcass weights for the Brahmans and buffalo were, respectively, 0.8 ($P<0.05$) and 0.5 ($P>0.05$). There was no significant difference between the fat depth of buffalo and that of Brahmans at the same carcass weight.

With older and heavier Brahman-cross and buffalo steers, the dressing percentage of buffalo was significantly less than that of Brahmans but the fat depth over the loin was significantly greater in buffalo (Robertson et al., 1983). The difference in dressing percentage is probably in part a result of the heavier hide of buffalo relative to carcass weight (Arganosa et al., 1973). Reported differences in subcutaneous fat depth and carcass fat content between groups of buffalo and cattle may depend on the carcass weights at which the comparisons were made as well as the breeds of the two genera that were used.

As in our previous investigation, the fat color of the buffalo was uniformly white whereas that of the cattle varied from pale cream to yellowish.

ST muscles

The results are shown in Table 1. Tenderstretching had little effect except that it significantly increased ST sarcomere length values in both species. Over both treatments peak shear force and peak force minus initial yield force values were less for beef than for buffalo. These differences were significant for all treatment comparisons except for the peak shear force values of the Achilles tendon hung sides, where the difference was close to significance.

The greater peak force minus initial yield force values of buffalo ST samples were believed to reflect an increased contribution of connective tissue to toughness (Bouton et al., 1977). ST muscles have a moderately high collagen content and sarcomere lengths of at least 2.0 μm in both normally hung and tenderstretched sides. These factors would result in more consistent peak-force minus initial yield differences than may occur in muscles with shorter sarcomeres and less collagen.

The adhesion values were not significantly different but there were significant differences in the way the samples pulled apart during the adhesion measurement since the "work done" values for the samples from buffalo were 25% ($P<0.001$) greater than those from the beef. Adhesion values are also believed to be an index of connective tissue strength (Bouton and Harris, 1972). The greater "work done" values for buffalo muscles recorded during adhesion measurements also indicated a greater connective tissue contribution to toughness in these animals. Over both hanging treatments cooking loss values did not differ between species.

LD muscles

The results for LD muscles are shown in Tables 2 and 3. The WB peak shear force values for the buffalo were significantly greater than for the equivalent beef samples although there was no significant species difference for initial yield force values. The peak force minus initial yield force values were also significantly ($P<0.001$) greater for buffalo (1.48 vs 0.58 kg). The Instron compression and cooking loss values were significantly greater for the buffalo samples. Sarcomere length values indicated that hanging from the sacrosciatic ligament stretched LD muscles significantly more in beef than in buffalo carcasses. Ultimate pH values did not differ significantly between species.

The taste panel results showed that, with or without tenderstretching, the panel rated buffalo meat as tougher, although after tenderstretching LD muscles from neither were rated overly tough. From the juiciness point of view there were no species differences but tenderstretched samples were significantly juicier.

PM muscles

The results obtained are listed in Tables 4 and 5. There were no differences between species in sarcomere lengths. Tenderstretching had shortened the muscles but not to the point where shear force values (and toughness) were adversely affected and significantly decreased initial yield force values for the buffalo but not for the beef, a similar result to that obtained in an earlier experiment (Robertson et al., 1983). Cooking losses were significantly increased by tenderstretching, but there were

Table 6—Mean Warner-Bratzler shear force results obtained for samples of buffalo and Brahman-cross semimembranosus muscles cooked at 60° or 80°C for 1 hr and showing the effect of hanging carcass sides from the Achilles tendon (AT) or sacrosciatic ligament (TS)

Parameter measured	Species	Hanging method/cooking temperatures				LSD[a]		
		AT60	AT80	TS60	TS80	A	B	C
WB peak shear force (kg)	Buffalo	5.06	7.03	6.40	6.84	1.29	1.13	1.09
	Beef	5.34	6.86	5.65	5.07			
WB initial yield force (kg)	Buffalo	2.98	4.82	2.46	3.90	0.94	0.79	0.84
	Beef	3.47	4.94	2.30	3.68			
WB peak – initial yield force (kg)	Buffalo	2.08	2.21	3.94	2.94	0.99	1.04	0.90
	Beef	1.87	1.92	3.35	1.39			

[a] Least significant difference at P = 0.05 for between species within hanging treatments and cooking temperatures (A), between cooking temperatures within species and hanging treatments (B) and between hanging treatments within species and cooking temperature (C).

Table 7—Mean Hunter opponent color values (L, a and b) of surfaces of raw samples of semitendinosus (ST) and semimembranosus (SM) muscles from buffalo and beef (Brahman-cross) steers

	Species/Muscle				LSD[a]	
	Buffalo		Beef			
Parameter	SM	ST	SM	ST	A	B
L	28.5	32.4	31.3	37.5	1.8	1.0
a	16.6	15.1	17.5	14.8	0.8	0.6
b	7.8	8.7	9.6	10.5	1.0	0.5

[a] Least significant difference between means at P = 0.05 for between species within a muscle (A), between muscles within a species (B).

Table 8—Mean Hunter opponent color values (L, a and b) measured on surfaces of raw buffalo and beef semimembranosus muscles

	Species/Sample treatment				LSD[b]	
	Buffalo		Beef			
Parameter	Normal[a]	Normal + 24[a]	Normal	Normal + 24	A	B
L	28.5	27.4	31.3	31.4	1.3	0.4
a	16.7	13.0	17.5	15.0	0.7	0.6
b	7.9	6.6	9.6	8.9	0.7	0.3

[a] (a) freshly cut and bloomed for 1 hr at room temperature (Normal) and (b) exposed for 24 hr at 0–1°C to moist air (Normal + 24).

[b] Least significant difference at P = 0.05 between species within a sample treatment (A) and between sample treatment within a species (B).

no species differences. Peak shear force values were significantly increased ($P<0.05$) when Brahman, but not buffalo, sides were tenderstretched. However, peak force minus initial yield values, over both hanging treatments, were not significantly different.

The taste panel results (Table 5) show that buffalo had significantly less acceptable flavor and acceptability than the beef samples although the differences were not large. There were no apparent differences in tenderness or juiciness between species.

SM muscles

The shear force results obtained for the muscle samples cooked at 60° or 80°C for 1 hr are listed in Table 6. Peak shear force values for tenderstretched SM muscles from the buffalo were significantly greater than those of corresponding muscles from the Brahman crosses, whereas there was no species difference in peak shear force values of muscles from Achilles tendon-hung sides. Averaged over-all treatments, initial yield force values did not differ significantly between species. Initial yield force values were significantly greater when samples were cooked at 80°C rather than 60°C. Over both cooking and hanging treatments peak force minus initial yield values were significantly greater for buffalo samples. However, within carcass hanging treatments, this difference was significant only for tenderstretched samples.

The greater peak force minus initial yield values for buffalo indicated that the contribution of connective tissue to toughness was greater in buffalo muscles. A similar result was found with the ST and LD muscles examined in this and in a previous experiment with older animals (Robertson et al., 1983). The extent of the between species, within muscle, differences in peak force minus initial yield values decreased as the mean peak force minus initial yield values for a muscle, over both species, decreased ($r = 0.77$).

Color measurements

Results of color measurements are shown in Tables 7 and 8. Although both Hunter Opponent colors values (L, a and b) and C.I.E. Tristimulus values (Y, X and Z) were recorded, only the former are discussed. The SM muscles were significantly darker than ST muscles, and beef muscles were lighter than similar buffalo muscles. These differences are similar to those obtained previously (Robertson et al., 1983) for samples from 51 month-old animals. A comparison of the results from this and the earlier work showed that muscles from older animals were darker than those from these younger animals (L values were less).

Measurements on the color of muscle surfaces after exposure to air for 24 hr were taken because, in the earlier experiment, with samples from older animals, we believed that buffalo meat darkened more than beef when exposed to moist air for 24 hr at chill temperatures. Results (Table 8) from the present measurements confirmed this previous, subjective assessment. Exposure to moist air at 0–1°C decreased the lightness (L), a and b values of buffalo SM muscles significantly more than it did those of beef SM muscles. This darker color was not due to differences in ultimate pH of muscles but probably to the greater pigment concentration in buffalo muscles than in beef muscles (Arganosa et al., 1973).

CONCLUSIONS

MUSCLES from younger buffalo were sufficiently darker than similar muscles from beef animals of the same age to disadvantage them in retail displays in locations where purchasers are used to buying only beef.

Because Warner-Bratzler initial yield force values generally did not differ much between the species, the myofibrillar contribution to toughness was similar in the four beef and buffalo muscles examined. The greater Warner-Braztler peak force values for those muscles (ST, LD and SM) which, in beef animals, have a reasonably high connective tissue content resulted from a greater contribution of connective tissue to toughness in buffalo than in beef muscles. Warner-Bratzler peak shear force values of PM muscles (low connective tissue content) did not differ between species consistent with this conclusion.

Although species differences in tenderness were not large, they were consistent. Tenderstretching resulted in the LD muscles of both species being acceptably tender. The flavor and overall sensory acceptability scores for buffalo PM muscles were slightly, but significantly, less than for beef.

In the present study the husbandry, transportation and preslaughter treatment of animals was considered to be such that preslaughter stress was minimal. It is possible that, if the comparison was made between buffalo and Brahmans that were not used to being handled and/or were transported long distances to slaughter, the present results might not be applicable, particularly if the meat were not tenderstretched or effectively electrically stimulated; however, the greater contribution of connective tissue to the toughness of buffalo would persist.

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Proportion of Collagen Types I and III in Four Bovine Muscles Differing in Tenderness

D. E. BURSON and M. C. HUNT

ABSTRACT

Intramuscular collagen containing both endomysial and perimysial tissue was extracted from four bovine muscles, analyzed for collagen types I and III, and related to total muscle collagen and meat tenderness. Isolated collagen was digested with cyanogen bromide and the proportion of collagen types I and III determined from densitometric scans of peptides resolved by SDS-PAGE. Total collagen was different ($P<0.05$) for each muscle. However, only the longissimus had more ($P<0.05$) type III collagen than did the biceps femoris, semimembranosus, and semitendinosus muscles. Percentage type III was correlated with sensory panel connective tissue amount (0.48) and overall tenderness (0.48) scores for the longissimus and biceps femoris muscles.

INTRODUCTION

THE CONCEPT that different muscles from animals of similar age vary greatly in tenderness is well established (Ramsbottom and Strandine, 1948). Connective tissue is a major factor affecting tenderness, since total collagen (Mitchell et al., 1928), heat soluble collagen (Hill, 1966), and the degree of collagen crosslinking (Shimokomaki et al., 1972) influence tenderness. However, skeletal muscle is now known to contain at least four different types of collagen (Sims and Bailey, 1981). How these types affect tenderness is uncertain.

Types I and III collagen appear to be the predominant types in normal muscle (Light and Champion, 1984). When type III is expressed as a percentage of total types I and III collagen, more tender muscles may have a lower percentage of type III in perimysial and endomysial collagens than less tender muscles (Bailey et al. 1979). In a study involving only perimysial collagen, Light and Bailey (1983) reported that the longissimus had a lower percentage of type III collagen than five other muscles that were rated both less tender and more tender than the longissimus. In later studies using endomysial or perimysial collagen from the same six muscles, no differences in the percentage of type III collagen was noted among muscles (Light et al., 1984a, b).

The purpose of this study was to evaluate the proportion of types I and III collagen in the connective tissues of four bovine muscles varying in tenderness.

MATERIALS & METHODS

LONGISSIMUS (LD), biceps femoris (BF), semimembranosus (SM), and semitendinosus (ST) muscle samples were removed from eight Simmental bulls that had been fed a high concentrate corn diet for 9.7 mo before slaughter at 17.4 mo of age and had an average USDA yield grade of 1.8 and a USDA quality grade of high Good. Samples were collected 7 days after slaughter and stored at −20°C. Additional LD and BF steaks for sensory panel and Warner Bratzler shear analyses also were collected, packaged, and stored at −20°C.

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Isolation of intramuscular collagen

Muscle samples were prepared for connective tissue isolation by removing the outer fat and epimysium before the muscle was minced into approximately 1 cm cubes. Ten grams minced muscle were homogenized with a Waring Blendor and intramuscular collagen (IMC) was isolated as described by Fujii and Murota (1982). In addition, IMC was homogenized in distilled water, washed briefly with 2% (w/v) sodium dodecyl sulfate (SDS) as suggested by Laurent et al. (1981), rinsed with distilled water, freeze-dried, and stored desiccated at 4°C for not more than 90 days. Recovery of hydroxyproline in the isolated IMC was 85–90% of the total muscle hydroxyproline.

Cyanogen bromide digestion of IMC.

Cyanogen bromide peptides were prepared in a manner similar to Light and Bailey (1979). Twenty milligrams IMC from each muscle sample were suspended in 2 mL 70% formic acid, 40 mg cyanogen bromide (CNBr) were added and the samples were incubated in a sealed digestion vessel at 30°C for 4 hr. If after 3 hr insoluble material was still present, an additional 20 mg CNBr was added and the digestion continued for two additional hours. Digestion was stopped by adding 10 volumes distilled water and the sample was evaporated to the original volume in vacuo at 30–35°C. Ten volumes distilled water were again added and the sample was lyophilized.

Resolution and quantitation of cyanogen bromide peptides.

Intramuscular collagen CNBr peptides were dissolved (5 mg/mL) by incubating at 60°C for 20 min in a solution of 2% (w/v) SDS, 0.125M Tris-HCl pH 6.8, 0.7M 2-mercaptoethanol, 1% (v/v) glycerol, 0.002% (w/v) bromophenol blue.

The CNBr peptides were electrophoretically resolved on 1.5 mm slab SDS-polyacrylamide gels containing 9.5% (w/v) acrylamide, 0.32% (w/v) N,N'-methylenebisacrylamide, 0.2% (w/v) SDS. Buffer systems and stacking gel concentrations were those described by Laemmli (1970). Electrophoresis was conducted at 25 mamps for 4–4½ hr at 4°C.

Gels were stained in 250 mL of a freshly made solution of 0.05% (w/v) Coomassie brilliant blue R-250, 50% (v/v) methanol and 9.2% (v/v) acetic acid for 4 hr using gentle shaking at room temperature. Destaining of gels was performed for 20 hr in several changes of 250 mL 10% (v/v) methanol, 7.5% (v/v) acetic acid with gentle shaking at room temperature. Immediately after destaining gels were scanned with a densitometer at 530 nm. Peak area of peptides α1(I)CB8 and α1(III)CB8 was determined using an integrator for the quantitation of type III collagen expressed as a percentage of total types I and III collagen as described by Light (1982). The α1(III)CB5 peptide used by Light and Champion (1984) to quantitate type III was not used in this study since another band that migrated slightly faster than α1(III)CB5 interfered with densitometric measurements.

Preparation of Type I and III Standards

Types I and III collagen were prepared from 5 mo fetal calf skin by fractional precipitation of pepsin-soluble extracts at 1.6M NaCl for type III and 2.5M NaCl for type I (Miller and Rhodes, 1982). Cyanogen bromide peptides were prepared for each type, resolved on 9.5% SDS-polyacrylamide gels, and scanned with a densitometer as described above. The α 1(I)CB8 and α1(III)CB8 peptides had essentially linear staining characteristics up to a total protein concentration of 75 μg per gel track (Fig. 1). In the linear portion of the curve, each peptide on any one gel bound approximately equal amounts of stain (peak area) per unit of peptide, which agrees with Light (1982).

To calculate type III as a percentage of the total types I and III collagen, peak areas were adjusted for the number of alpha chains and

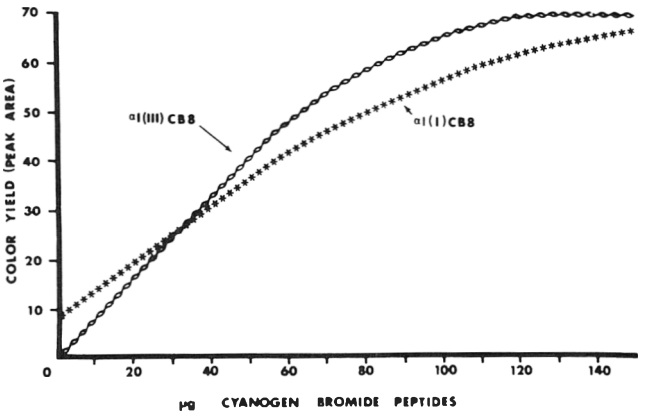

Fig. 1—Color yield in arbitrary integrator units of Coomassie brilliant blue stain of collagen peptides α1(I)CB8 and α1(III)CB8 relative to the amount of cyanogen bromide peptide digest loaded on each sodium dodecyl sulfate-polyacrylamide gel track.

the peptide size of 279 residues for α1(I)CB8 and 180 residues for α1(III)CB8 (Galloway, 1982) using the following formula:

$$\% \text{ Type III} = \left[\frac{\text{peak area } \alpha1(\text{III})\text{CB8}/180}{(\text{peak area } \alpha1(\text{I})\text{CB8}/279 \times 3/2) + (\text{peak area } \alpha1(\text{III})\text{CB8}/180)}\right] \times 100$$

Total collagen evaluation.

Minced muscle (4g) from each sample was homogenized with a Polytron and hydrolyzed in 6N HCl for 6–12 hr in an autoclave at 125°C. Hydroxyproline was determined by a modification of the Bergman and Loxley (1963) rapid procedure, in which 2 mL Ehrlich's reagent was used and the final color solution as not dilute. A conversion factor of 7.25 (Goll et al., 1964) was used to express hydroxyproline as total collagen.

Sensory evaluation

Longissimus and BF steaks for sensory evaluation were thawed for 16 hr at 4°C and oven-broiled at 166°C to an internal temperature of 70°C. Cores 1.3 cm in diameter were removed from each steak and were evaluated by an eight member trained sensory panel (AMSA, 1978). Connective tissue amount, myofibrillar tenderness, and overall tenderness were evaluated using an eight point scale (1 = abundant connective tissue or extremely tough; 8 = no connective tissue or extremely tender). Myofibrillar tenderness is the initial resistance of the myofiber to chewing relative to both the quantity and quality of connective tissue residue remaining after chewing. Overall tenderness is an overall impression of tenderness considering both the connective tissue amount and myofibrillar tenderness evaluations.

Steaks for shear force analysis were cooked according to the procedures described for the sensory panel and then cooled at room temperature for 2 hr before 1.3 cm cores were removed. Shear force was determined with a Warner-Bratzler shearing device for eight cores from each LD and BF steak.

Statistical analysis

Data were analyzed by analysis of variance and means were separated by Duncan's multiple range test using the Statistical Analysis Systems package (SAS, 1982).

RESULTS & DISCUSSION

SENSORY PANEL SCORES for muscle fiber tenderness were similar ($P>0.05$) for the LD and BF (Table 1). However, the BF had more detectable connective tissue, lower overall tenderness scores, and greater Warner-Bratzler shear values than the LD muscles.

Total collagen per gram fresh tissue (Table 1) was different ($P<0.05$) for each muscle, with the LD < SM < ST < BF. The amount of IMC extracted for each muscle followed a pattern similar to that of total muscle collagen, since the dry weight of isolated IMC per gram fresh tissue was also different ($P<0.05$) with LD = SM < ST < BF. The dry weight of IMC per unit of total muscle collagen was not different ($P>0.05$) for the four muscles, suggesting that the percentage of collagen extracted was similar for each muscle.

Figure 2 shows the CNBr peptides used for quantitation on SDS-polyacrylamide gels. Percentage type III collagen (Table 1) was greater ($P<0.05$) for the LD (33.6%) than from the SM (28.9%), ST (28.3%), and BF (27.8%). This indicates that a more tender cut (LD) with less connective tissue has a higher percentage type III collagen than muscles that are less tender (BF) and contain more connective tissue (SM, ST, BF). However, there were no differences ($P>0.05$) in the percentage of type III for the ST, SM, and BF, all muscles of the rear appendage. These muscles also varied in tenderness (Ramsbottom and Strandine, 1948) and were different ($P<0.05$) in the amount of connective tissue (Table 1). Percentage type III collagen was correlated ($P<0.05$) with sensory panel connective tissue amount (0.48) and overall tenderness (0.48) scores for the LD and BF muscles.

Light and Bailey (1983) reported that the perimysium of the psoas major had a greater percentage of type III collagen than the LD, which is characteristically less tender than the psoas major. Yet in four other less tender muscles, the ST, pectoralis profundus, gastrocnemius and sternomandibularis, the percentages of type III perimysial collagen were similar to that of the psoas major. In studies involving the same six muscles, no differences were noted for percentage of type III endomysial or perimysial collagen (Light et al., 1984a, b). Bailey et al. (1979) reported that for combined endomysial and perimysial connective tissues, the percentage type III for the LD (30%) and the ST (31%), two of the more tender, lower connective tissue muscles, was less than the percentage type III for the

Table 1—Collagen characteristics, sensory evaluation[a] and Warner Bratzler shear values of the longissimus, biceps femoris, semimembranosus, and semitendinosus muscles

Trait	Muscle			
	Longissimus	Biceps femoris	Semimembranosus	Semitendinosus
Sensory traits				
Myofibrillar tenderness	5.8	5.2		
Connective tissue amount	6.2[c]	3.9[b]		
Overall tenderness	5.9[c]	4.3[b]		
Warner-Bratzler shear, kg	3.4[b]	5.0[c]		
Whole muscle tissue				
Total collagen, mg/g	4.92[b]	11.11[e]	6.85[c]	9.40[d]
Isolated intramuscular collagen				
Dry weight, mg/g muscle	10.5[b]	21.7[d]	13.3[b]	17.5[c]
Dry weight, mg/mg collagen	2.0	1.9	1.9	1.8
Percentage type III collagen	33.6[c]	27.8[b]	28.9[b]	28.3[b]

[a] Scores based on an eight point scale for each factor (1 = abundant connective tissue or extremely tough; 8 = no connective tissue or extremely tender).
[b,c,d,e] Means within a row with different superscripts are different ($P<0.05$).

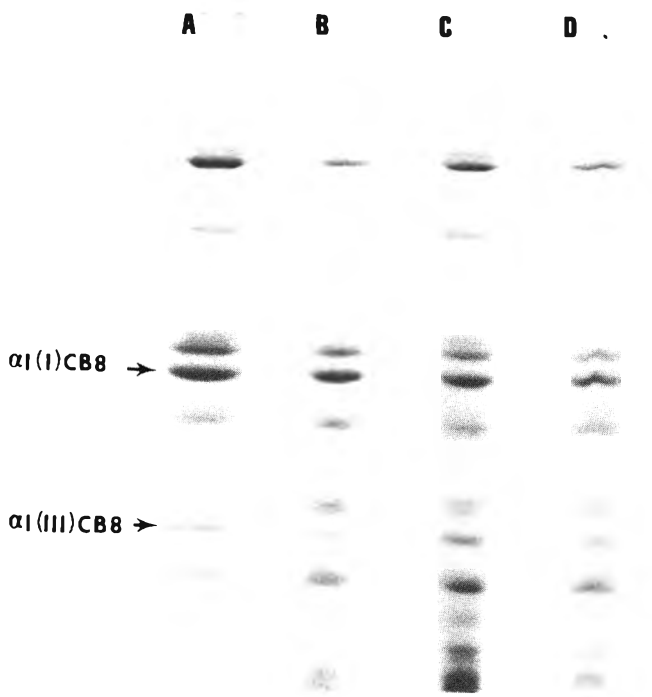

Fig. 2—Resolution of cyanogen bromide peptides of intramuscular collagen isolated from (a) longissimus, (b) biceps femoris, (c) semimembranosus, and (d) semitendinosus muscles on sodium-dodecyl sulfate-polyacrylamide gels (9.5%).

sternomandibularis (40%) and carpi radialis (43%) two of the less tender, higher connective tissue muscles.

Even though this study indicates that muscles from bulls with a high percentage of type III collagen are more tender, the relationship of the proportion of type I and III intramuscular collagens and tenderness of different muscles appear to be variable. Other factors such as intermolecular crosslinking and fiber size of the different collagen types may play a greater role in tenderness of muscle than the relative proportion of types I and III collagen.

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Role of Fimbriae and Flagella in the Attachment of *Salmonella typhimurium* to Poultry Skin

H. S. LILLARD

ABSTRACT

The role of fimbriae and flagella in the mechanism of attachment of *S. typhimurium* to poultry skin was examined by using cells with (1) type 1 fimbriae (flagella present), (2) cells from which fimbriae and flagella were removed by physical means, (3) cells grown at 18°C which produced relatively thick, mannose-sensitive type 1 fimbriae only, and (4) cells grown in the presence of D-mannose which produced thin, mannose-resistent fimbriae. When broiler skin was immersed in the above cell suspensions, *S. typhimurium* cells adhered firmly and equally well in all instances except that fewer cells attached when grown in the presence of D-mannose. When fimbriated and nonfimbriated cells were spread on broiler skin, thus avoiding effects of water uptake, cells attached, but in lower numbers than when skin was immersed in a cell suspension. It was concluded that bacterial attachment to poultry skin is a complex phenomenon which involves mechanisms other than fimbriae, flagella or water uptake, though a combination of these and other factors may be involved.

INTRODUCTION

BRINTON (1965) and Duguid et al. (1966) showed that there are several types of fimbriae among the *Enterobacteriaceae*, and these are distinguishable by their morphology and hemagglutinating properties. The most common type is named type 1 fimbriae. Duguid et al. (1966) pointed out that most genera and species, including *Salmonella typhimurium*, exhibit adhesive properties which are commonly due to type 1 pili. The adhesive properties of type 1 pili are inhibited by D-mannose.

Jones et al. (1980) concluded that type 1 pili are not the main adhesins involved in the attachment of *S. typhimurium* to HeLa cells. Working with a type 1 (mannose-sensitive) piliate strain, Jones et al. (1980) found that D-mannose did not prevent salmonellae from attaching to HeLa cells. However, they found that homogenization prevented the attachment of *S. typhimurium* to HeLa cells. They assumed that homogenization released the bacterial cell adhesin which facilitates attachment to the HeLa cell receptor thus preventing subsequent attachment. Jones et al. (1980) demonstrated the presence of a cell-bound D-mannose resistant hemagglutinin (MR-hemagglutinin) which they suspected was responsible for the attachment of *S. typhimurium* to HeLa cells. The MR-hemagglutinin was produced in cultures grown at 37°C but not in cultures grown at 18°C. The former cultures attached to HeLa cells whereas the latter did not.

Gaastra and De Graaf (1982) reviewed the role of host-specific adhesins in the attachment of noninvasive enterotoxigenic strains of *Escherichia coli* to mucosal epithilium. They stated that the currently known surface antigens with adhesive properties (pili) have been identified as nonflagellar, filamentous, proteinaceous appendages on the bacterial cell surface. None of the adhesins of enterotoxigenic *E. coli* studied to date are produced at 18°C, except type 1 fimbriae which are produced at all temperatures. Jones et al. (1980) had reported that the MR-hemagglutinin of *S. typhimurium* was not produced at 18°C.

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Several authors reported that flagellated bacteria attach more readily than nonflagellated bacteria to poultry and meat surfaces (Notermans and Kampelmacher, 1974; Butler et al., 1979; Farber and Idziak, 1984). Others were not able to confirm these results (McMeekin and Thomas, 1978; Lillard, 1985).

This study was undertaken to determine if the attachment of *S. typhimurium* to poultry skin could be affected by the absence of water uptake, the presence of D-mannose in the growth medium, incubation of the culture at 18°C, or by homogenizing the cells to remove fimbriae and flagella prior to exposing poultry skin to the cell suspension.

MATERIALS & METHODS

S. TYPHIMURIUM was selected because it is the species of *Salmonella* most frequently isolated from raw broiler carcasses; the strain used in this study was isolated from this source. *S. typhimurium* cells used in the attachment studies were subjected to the following four treatments:

Experiment 1. Cells were grown in nutrient broth prepared by the method of Duguid and Gillies (1957) and incubated at 37°C for 24 hr. Cells from a second serial broth culture were used in these experiments. These growth conditions are conducive to the development of fimbriae and flagella on the cell surface (Duguid et al., 1966).

Experiment 2. Cells were grown in nutrient broth (Difco) and incubated at 18°C for 36 hr. Cells from the second serial broth culture were used in these experiments. This treatment was expected to inhibit development of all but type 1 fimbriae.

Experiment 3. Cells were grown in nutrient broth (Duguid and Gillies, 1957) and incubated at 37°C for 24 hr. These cells were then defimbriated and deflagellated by the following procedure prior to use in the attachment experiments: Cells were harvested by centrifuging at 1600 × *g* for 10 min at 9°C, the supernatent was decanted and the cells resuspended in 65 mL of 0.85% saline. This cell suspension was blended (Sorvall OMNI-Mixer) in an ice bath at high speed (#10 setting) for 10 min. Temperature of the cell suspension during homogenization was maintained at 8–13°C. The cells were then centrifuged at 60,000 × *g* for 10 min at 8°C (Sorvall RC-5B refrigerated centrifuge), washed twice with 0.85% saline and recentrifuged. The combination of high speed blending and high speed centrifugation resulted in cells with intact cell walls from which both flagella and fimbriae had been removed and retained in the supernatent fluid. The integrity of these cells was confirmed by their ability to reproduce normally and form fimbriae and flagella upon culturing as did the parent cells upon incubation in broth medium and as confirmed by transmission electron microscopy. The absence of fimbriae and flagella for the duration of the experiment (0.25 to 60 min) was confirmed by electron microscopy.

Experiment 4. Cells were grown at 37°C for 48 hr in nutrient broth (Difco) to which was added 0.5% D-mannose (Sigma). This treatment was expected to inhibit the formation of mannose-sensitive type 1 fimbriae.

Preparation of *S. typhimurium* cell suspension for attached experiments

A second serial broth culture grown in 1L of nutrient broth, as specified for each experiment, was inoculated with 2 mL of an 18–24 hr culture and incubated at the temperature specified above for each experiment. The cells were then harvested by centrifugation (6500 × *g* for 15 min at 9°C), washed twice in 0.85% saline and resuspended in 2L of 0.85% saline (pH 6.2–6.5), except for Experiment 3 in which cells were defimbriated and deflagellated before preparing the final cell suspension.

Attachment experiments No. 1–4

Skin from commercially soft-scalded (52°C for 2.25 min), defeathered but uneviscerated broilers was used throughout the experiments. Water uptake by poultry skin does not occur during scalding. Therefore, these broilers were used in order to obtain skin which had not been altered by water uptake. The same location on breasts was used to excise 12 cm^2 pieces of skin which were added to the appropriate cell suspension and stirred magnetically. Skin pieces were removed following immersion in the cell suspension for 0.25, 30, and 60 min, and excess fluid was drained (30 sec), the skin was rinsed with a salmonella-free cold tap water spray at 50 psi for 1 min as described by Lillard (1985). Cells which did not wash off were considered attached. Skin samples (and a control which had not been immersed in the cell suspension) were then homogenized in a Colworth Stomacher (Cooke Laboratory Products, Alexandria, VA) in 10 mL of 0.85% saline for 1 min. Samples of the homogenate (0.1 mL each) were serially diluted then spread on duplicate BG sulfa agar plates (Difco) for enumeration and incubated at 35°C for 24 hr.

Experiments were repeated four times. Results reported are the means from four experiments.

Spread inocula. A *S. typhimurium* cell suspension was prepared as above from a culture grown as in Experiment 1. In order to avoid changes in the microtopography of the skin attributed to water immersion (Thomas and McMeekin, 1984), an inoculum of 0.1 ml was spread with a hockey stick on the whole skin of each broiler half-breast which was then covered with foil (without skin contact) to prevent drying. Breasts were held for 0.25, 30, and 60 min; the whole excised skin was then spray rinsed at 50 psi, homogenized, and *S. typhimurium* cells enumerated as described above. Control samples were not rinsed prior to sampling in order to obtain an accurate recovery count of the spread inoculum.

Sample preparation for transmission electron microscopy. *S. typhimurium* cells grown as described in Experiments 1–4 were centrifuged (1600 × *g* for 15 min) and washed three times with distilled water (10 mL each time) to remove salts that may be present in culture medium. The washed cells were resuspended in 2 mL distilled water. A drop of this cell suspension was placed on a 300 mesh Formvar resin-coated grid (Ted Pella, Inc., Tustin, CA), prepared as described by Dawes (1981). The drop was allowed to stand 2 min, excess was blotted off with filter paper and a drop of 1% phosphotungstic acid was added, the excess removed with filter paper after 1 min (Dawes, 1981). The grid was then examined by transmission electron microscopy (JEOL Model JEM-100 CX-11; accelerating voltage 80 Kv).

RESULTS & DISCUSSION

Duguid et al. (1966) showed that most strains of *Salmonella* serotypes are fimbriated and that all fimbriated strains but one were mannose-sensitive. Furthermore, they showed that most or all fimbriated strains of *Salmonella* vary between a fimbriate and nonfimbriate phase. A pure culture of fimbriated *Salmonella* will contain cells with flagella and no fimbriae, flagella and fimbriae, fimbriae only and some with neither. Fimbriation usually appears in at least the second culture, diminishes greatly during the logarithmic growth phase, but reaches the original proportion after 24-48 hr incubation. The electron micrographs shown in Fig. 1 represent the predominant form of the cells as used in the various experiments.

Cells grown at 35°C for 24 hr were predominantly flagellated and strongly fimbriated (Fig. 1a). A large number of these cells adhered to poultry skin immersed in this cell suspension for 0.25, 30, and 60 min with a larger number adhering after 30 and 60 min than within 0.25 min (Table 1, Experiment 1). A large number of cells adhered immediately after 15 sec of immersion, but a greater number of cells adhered to the skin following 30 to 60 min immersion times. These results are in agreement with those reported by Thomas and McMeekin (1984) and Lillard (1985).

Cells grown at 18°C were predominantly fimbriated but not flagellated (Fig. 1b), although some cells were flagellated. Gaastra and De Graaf (1982) reported that all adhesins of enterotoxigenic *Escherichia coli* strains studied so far are not produced at 18°C except for type 1 fimbriae which appear to be produced at all temperatures. Type 1 fimbriae were found in all but 45 of 1184 fimbriated strains of salmonellae (Duguid et al., 1966). These fimbriae are relatively thick and bear the mannose-sensitive haemagglutinin (MS adhesion) described by Duguid (1964). Fimbriae produced at 18°C were relatively thick (Fig. 1b). These cells also adhered readily to poultry skin immersed in the cell suspension for 0.25, 30, and 60 min and a greater number adhered following 30 and 60 min immersion times than in the initial 0.25 min exposure (Table 1, Experiment 2). Even though the numbers adhering were between 0.5 and 1 $\log_{10}$ lower than for cells grown at 35°C, this difference was not considered important considering the 2 $\log_{10}$ difference in the cell numbers of the suspensions in which the skin was immersed. Notermans and Kampelmacher (1974) and McMeekin and Thomas (1978) showed that the greater the concentration of cells in the suspension medium the more cells attached to poultry skin.

When flagella and fimbriae were physically removed, cells with intact cell walls were obtained (Fig. 1c). However, the absence of flagella and fimbriae seemingly had no effect on the ability of the cells to adhere to poultry skin (Table 1, Experiment 3). Jones et al. (1980) found that homogenization prevented the attachment of *S. typhimurium* cells to HeLa cells. They showed that homogenization released material from the surface of adhesive, nonpiliated cells which prevented the attachment of salmonellae to HeLa cells. However, homogenization did not prevent the attachment of *S. typhimurium* to poultry skin (Table 1, Experiment 3). This would indicate that the mode of attachment of *S. typhimurium* to poultry skin either involves a different mechanism than is involved in their attachment to HeLa cells or may be due to a difference in strains. An interaction between cell wall components and receptor sites on poultry skin may account for the attachment of defimbriated and deflagellated cells. Consideration must, therefore, be given to the particular substrate being investigated to which bacteria might adhere. The fact that defimbriated and deflagellated cells adhered well to poultry skin immersed in the cell suspension confirms previous findings (Lillard, 1985) which showed that motile and nonmotile, gram positive and gram negative, fimbriated and nonfimbriated species of bacteria attached to poultry skin under similar experimental conditions.

When *S. typhimurium* cells were grown in broth containing D-mannose, fimbriation was sparse (Fig. 1d) and differed in appearance from fimbriae produced at 37°C (Fig. 1a). Type 1 fimbriae (Figs. 1a and 1b) are thicker and, for the most part longer or less fragile appearing than fimbriae produced in the presence of D-mannose (Fig. 1d) indicating that this strain of *S. typhimurium* is capable of producing more than one type of fimbriae. Duguid et al. (1966) described a "thin-type" mannose-resistant type 3 fimbriae found in almost all strains of *Klebsiella aerogenosa* and *Seratia marcescens*. Most of the strains also possessed type 1 fimbriae. Even though cells grown in D-mannose adhered to poultry skin immersed in the cell suspension (Table 1, Experiment 4), the number of cells adhering was lower than in the other experiments. Since cells with type 1 fimbriae (Table 1, Experiments 1 and 2) and defimbriated, deflagellated cells (Table 1, Experiment 3) adhered in larger numbers than cells grown in the presence of D-mannose, the role of type 1 fimbriae and cell wall components would bear further investigation.

Thomas and McMeekin (1984) suggested that changes in the microtopography of poultry skin due to water immersion, such as the formation of deep channels in the skin, may explain the mechanism of bacterial attachment to skin immersed in a cell suspension. In order to eliminate the influence of water uptake and the concomitant alterations in the microtopography of poultry skin, inocula of the *S. typhimurium* culture with and without fimbriae were spread lightly on breast skin. Fimbriated and defimbriated cells adhered to the skin and could not be washed off (Table 2). Cells spread on only the outer surface of the skin adhered in lower numbers than in experiments where excised skin was immersed in a cell suspension. The surface area on which cells were spread equalled or exceeded the sur-

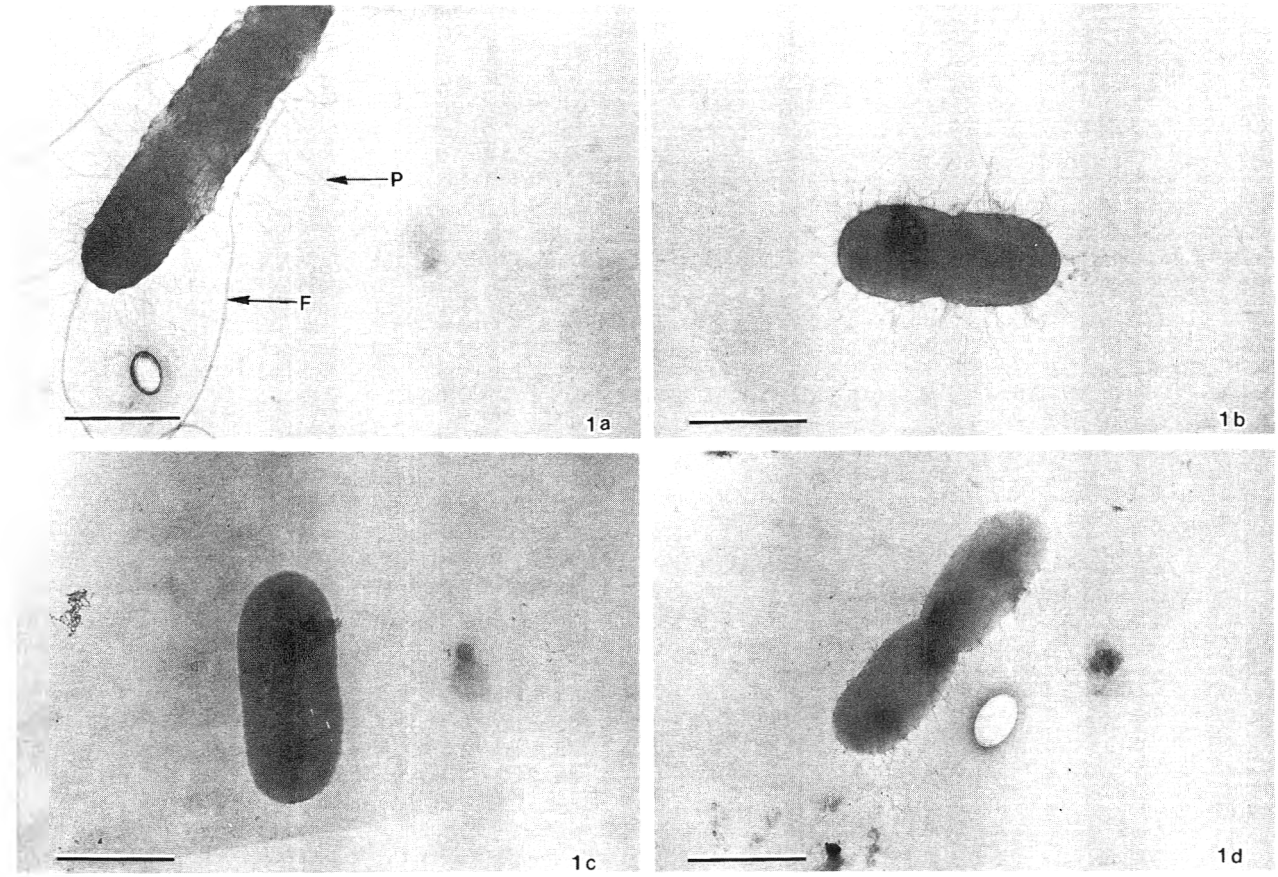

Fig. 1—Electron micrographs (×22,000) of S. typhimurium *cells (Bar = 1 μm): (a) Grown in nutrient broth (Duguid et al., 1966) and incubated at 37°C for 24 hr. P = pili; F = flagella. (b) Grown in nutrient broth (Difco) and incubated at 18°C for 36 hr. (c) Grown as in (a) but fimbriae and flagella removed by high-speed blending and centrifugation. (d) Grown in nutrient broth (Difco) containing 0.5% D-mannose.*

Table 1—Log_{10} colony forming units (cfu) recovered from broiler skin immersed in S. typhimurium *cell suspensions treated in four different ways*

Immersion time (min)	Log_{10} *S. typhimurium* cfu per 12 cm^2 skin[a]			
	Expt 1	Expt 2	Expt 3	Expt 4
0.25	7.25	6.81	6.91	5.19
30	8.27	7.27	8.14	5.80
60	7.98	7.54	8.51	5.81
Control	N.D.[b]	N.D.	N.D.	N.D.
Immersion fluid	10^8 cells/mL	10^6 cells/mL	10^8 cells/mL	10^6 cells/mL

[a] Values reported are means from four experiments: Expt 1 = Cells incubated at 37°C for 24 hr; Expt 2 = Cells incubated at 18°C for 36 hr; Expt 3 = Cells from which fimbriae and flagella were removed; Expt 4 = Cells grown in nutrient broth containing 0.5% D-mannose. N.D. = None detected.

Table 2—Log_{10} colony forming units (cfu) recovered from breast skin on which was spread a S. typhimurium *cell suspension*

Sampling time (min)	Log_{10} *S. typhimurium* cfu on total skin of half-breast	
	Total cfu recovered[a] (cells with pili and flagella)	Total cfu recovered[b] (cells without pili or flagella)
0.25	4.36	4.41
30	4.65	4.48
60	4.59	4.95
Control[c]	6.10	6.06

[a] Values reported are means from four experiments. Cells prepared as in Expt. 1 (with pili and flagella).

[b] Values reported are means from six experiments. Cells prepared as in Expt. 3 (without pili and flagella).

[c] Not rinsed prior to sampling.

face area of both sides of a 12 cm^2 piece of skin. Therefore, the difference in level of attachment may be attributed to a difference in composition, and therefore, receptor sites, on outer and inner surfaces of the skin, or may be associated with actual water uptake by the skin which was immersed. Even though the level of cells adhering was lower than for immersed skin (Experiments 1-4, Table 1), a sufficient number adhered thus indicating that the mechanism of salmonellae attachment to poultry skin involves more than microtopographical changes due to water uptake or the presence or absence of fimbriae.

It seems likely that the phenomenon of bacterial attachment to poultry skin involves more than one mechanism. Type of fimbriae, flagella, cell wall components and water uptake may all exert some influence in the process of bacterial adhesion to this substrate, but it seems unlikely that any one of these factors is solely responsible for the adhesion of *S. typhimurium* to poultry skin.

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Factors Influencing Color of Dark Cutting Beef Muscle

W.R. EGBERT and D.P. CORNFORTH

ABSTRACT

Color of dark cutting beef rib muscle was measured as affected by treatment with rotenone or chilling in an oxygen rich atmosphere. Samples homogenized with the mitochondrial inhibitor, rotenone, or pH 5.0 buffer remained red for up to 1 hr. Control samples blended with water remained red when chilled but turned dark when held at room temperature. Thin slices of dark cutting beef muscle would turn red when chilled in air or oxygen to 3°C, or when chilled in oxygen to 14°C, but would turn dark when transferred from oxygen at 3°C to air at room temperature. Thus, dark cutting beef muscle will turn red if mitochondrial respiration is inhibited, allowing myoglobin at muscle surfaces to remain oxygenated.

INTRODUCTION

DARK CUTTING BEEF is characterized by a high postmortem pH, increased water binding capacity and sticky texture, and the fact that it will not bloom when exposed to air. Bloom is the development of a bright red color when meat is exposed to air, due to the oxygenation of myoglobin to form oxymyoglobin as the predominant surface pigment. Since dark cutting meat will not bloom when exposed to air, it is discounted at the retail level (Price and Schweigert, 1978). The high pH of dark cutting beef is only indirectly related to color. The dark color is more directly related to high mitochondrial respiration, which keeps oxymyoglobin concentration low. In normal meat, postmortem glycolysis reduces pH to 5.8 or lower which impairs mitochondrial oxygen consumption (Ashmore et al., 1972) and allows normal bloom on meat surfaces exposed to air. Lawrie (1958) found that mitochondrial cytochrome oxidase was more active at pH values above 6.0, characteristic of dark cutting beef muscle, and concluded that increased oxygen consumption of dark cutting meat could increase the concentration of deoxygenated myoglobin, thus resulting in the dark color. Ashmore et al. (1973) showed that dark cutting meat could be experimentally prevented by injecting the live animal with propranolol, a competitive inhibitor of epinephrine, thus preventing antemortem depletion of muscle glycogen.

Incidence of dark cutting beef may be experimentally reduced by use of drugs to reduce antemortem glycolysis or more practically, by use of handling conditions that reduce antemortem stress. However, there is currently no way to obtain bloom in meat from dark cutters. Cornforth and Egbert (1985) showed that normally dark pre-rigor muscle would turn bright red when blended with the mitochondrial respiratory inhibitor, rotenone, or when thin slices were chilled in an oxygen-rich atmosphere (Cornforth et al., 1985). The objective of this study was to treat dark cutting meat with rotenone or chill it in an oxygen-rich atmosphere, in an attempt to inhibit or reduce mitochondrial respiration and thereby experimentally obtain bloom in dark cutting meat.

MATERIALS & METHODS

VACUUM PACKAGED, dark cutting beef ribs from "A" maturity cattle (average muscle pH of 6.4 at 96 hr postmortem) were obtained from a local abbatoir and refrigerated until needed. All experiments were performed within 4–7 days of slaughter.

In the first set of experiments, 25g of dark cutting Longissimus dorsi muscle (equilibrated to 20°C for 1 hr) were blended for 30 sec in an Osterizer blender (Sunbeam Corp., Milwaukee, WI) with: (1) 0.2g rotenone plus 75 mL water; (2) 75 mL water; (3) 75 mL of 0.2M citrate buffer, pH 5.0; (4) 75 mL chilled (2°C) water.

Samples were then poured into Hunter Color sample containers, and color readings (Hunter Associates Lab, Inc., Reston, VA) were taken within 30 sec after blending, and every 10 min for 1 hr. Treatments 1–3 were held at 20°C between color readings, while treatment 4 was held on ice throughout. Sample pH was recorded before and after treatment. The Hunter Lab digital color difference meter (D25D2A) was standardized with the red (tomato color) standard plate (L = 25.9, a = 27.4, b = 13.1).

In the second set of experiments, dark cutting Longissimus dorsi muscle samples were thinly sliced (2 mm thickness) with a Commercial food processing machine (Berkle, Inc., LaPorte, IN), dipped in isotonic saline and placed on plastic wrap to prevent drying, then treated as follows: (1) in air, 3°C (on ice); (2) in air at 20°C; (3) in oxygen at 3°C; (4) in oxygen at 3°C for 30 min, then transferred to air at 20°C; (5) in oxygen at 14°C.

Samples were oxygenated as described by Cornforth et al. (1985). Briefly, the oxygenation apparatus consisted of an oxygen tank connected by rubber tubing to a large anaerobic jar that contained ice to a depth of about 5 cm. Samples were held under these conditions for 30 min, then Hunter color readings were taken initially and at 10, 20, 30, and 60 min.

In treatment 4 of the second set of experiments, samples were held in oxygen at 3°C for 30 min before initial Hunter color readings were taken. The sample was then held in air at 20°C, and further color readings were taken at the specified intervals. For treatment 5, samples were held at 14°C by placing them in the oxygenation chamber on the top of a 600 mL inverted beaker. For all oxygenated samples, a slow flow of oxygen was used to flush the sample chamber throughout the experiment. Muscle samples were inverted in Hunter color sample containers before taking color readings to ensure that samples were measured on the surface directly exposed to air or oxygen. After each reading, the sample was returned to its original position until the next measurement. After the last color reading, a portion of each sample was minced and sample pH determined with an Orion pH meter (Model 601 A, Orion, Cambridge, MA).

One-way and two-way analyses of variance were performed as described by Ryan et al. (1980) for pH and color data, respectively. Least significant difference (LSD) values were computed where appropriate (Neter and Wasserman, 1974).

RESULTS

MEAN HUNTER COLOR 'a' or redness values for dark cutting rib muscle homogenates are shown in Fig. 1. All homogenates were initially red, due to formation of oxymyoglobin and exhibited redness values of 14.9–15.3. Samples homogenized with rotenone (a mitochondrial respiratory inhibitor) or pH 5.0 citrate buffer remained red for 1 hr after blending. Dark cutting muscle homogenized with water and held at 3°C (on ice) after blending also remained red for 1 hr. However, similar samples held at room temperature became significantly darker ($p<0.01$), with a mean redness value of 8.3 by 1 hr after blending.

Mean 'L' (lightness) and 'b' (yellowness) values for sample homogenates are presented in Tables 1 and 2, respectively. The mean 'L' value of all homogenates decreased with time (Table 1). Samples blended with water and held at 20°C were

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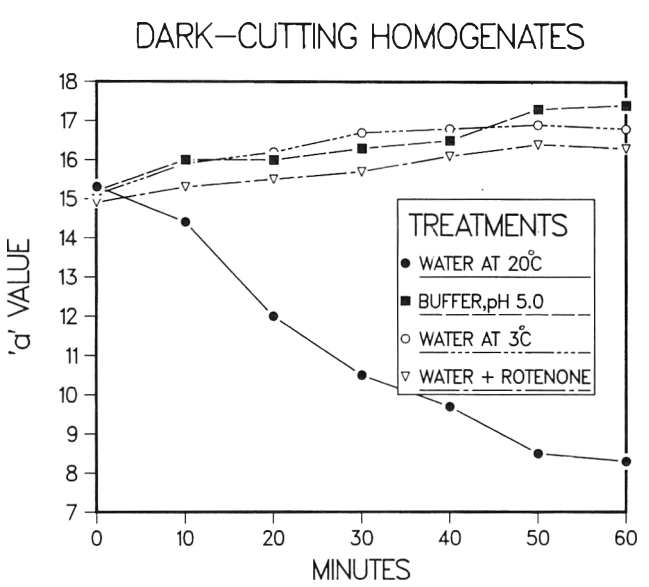

Fig. 1—Mean Hunter color 'a' or redness values for dark cutting rib muscle homogenates with time. Zero time is immediately after blending 25g muscle with 75 mL (1) deionized water, 20°C; (2) 0.2M citrate buffer at pH 5.0; (3) deionized water, 3°C; (4) deionized water plus 0.2g rotenone, respectively. Each point is the mean of four samples. The least significant difference (LSD) value = 1.43.

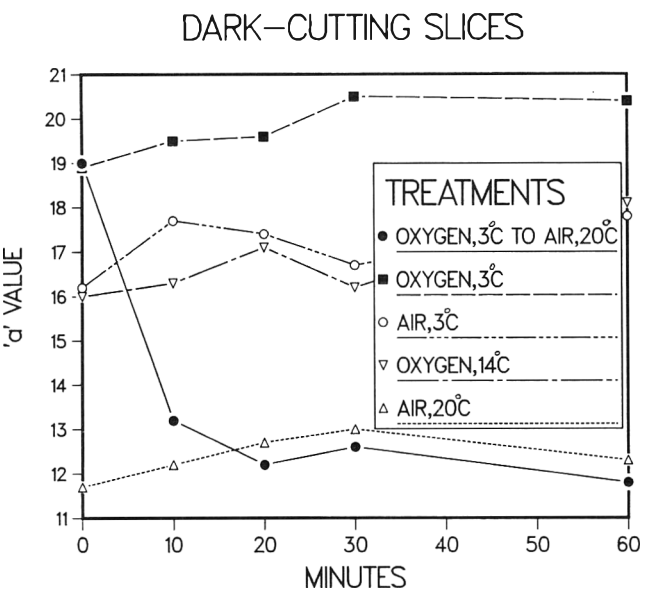

Fig. 2—Mean Hunter color 'a' or redness values for dark cutting rib muscle slices with time. Zero time equals 30 min after placing samples under the following treatment conditions: (1) oxygen, 3°C to air, 20°C; (2) oxygen, 3°C; (3) air, 3°C; (4) oxygen, 14°C; and (5) air, 20°C, respectively. Each point is the mean of four samples. LSD value = 1.48.

definitely darker than other treatments at all time intervals. The mean 'b' values of samples blended with water and held at 20°C also decreased significantly within 1 hr after blending compared to samples from all other treatments.

As expected, samples homogenized with pH 5.0 buffer had a significantly lower mean pH of 5.47, while all other samples had pH values above 6.3 (Table 3).

In the second set of experiments, thin (2 mm) slices of dark cutting longissimus muscle were subjected to various atmosphere and temperature conditions for 30 min prior to initial color readings. Both treatments in an oxygen atmosphere at 3°C (on ice) were bright red after 30 min and had significantly higher initial 'a' values than other treatments (Fig. 2). The samples held in oxygen at 3°C for 30 min, then transferred to air at 20°C for the duration of the experiment were bright red at the initial color reading but turned dark within 10 min of transfer to air at 20°C. These slices remained dark for all subsequent color readings (Fig. 2). Samples treated in air at 3°C or in oxygen at 14°C were also initially red in appearance with intermediate redness values. Samples in air at 20°C were initially dark with a significantly lower mean 'a' value of 11.7 (Fig. 2) and remained dark for the duration of the experiment. Redness of samples in air at 3°C, in oxygen at 14°C, or in oxygen at 3°C increased somewhat during the experiment. Among the three treatments, samples held in oxygen at 3°C had the highest mean redness value of 20.4 at 60 min, which was not significantly different from the redness value of samples in oxygen at 14°C (18.1) or in air at 3°C (17.8).

Mean Hunter color 'L' (lightness) and 'b' (yellowness) values for dark cutting rib muscle slices are presented in Tables 4 and 5, respectively. Lightness values did not vary significantly over time for any treatment or among treatments at any give time (Table 4).

Yellowness values did vary significantly with time within treatment and among treatments at a given time (Table 5). In general, trends in yellowness values were similar to those previously discussed for redness values. No significant differences were observed among treatments for pH values. All dark cutting rib muscle slices had a pH above 6.25, with a mean pH value of 6.28 (Table 6).

Muscle slices held in air at 3°C or in oxygen at 14°C exhibited typical post-rigor beef color, with 'a', 'b', and 'L' values of about 16, 9, and 26, respectively. Slices held in oxygen at 3°C were somewhat brighter red than normal post-rigor beef, with 'a', 'b', and 'L' values of 20, 10, and 27, respectively. Slices held in air at 20°C were much darker than normal post-rigor beef, with 'a', 'b', and 'L' values of 12, 6.6, and 23 (Fig. 2, Tables 4-5).

DISCUSSION

THIS IS apparently the first demonstration that dark cutting meat will bloom under appropriate conditions. Mitochondrial inhibition by rotenone, low pH, or chilling increased the redness of dark cutting beef muscle homogenates, as did chilling thin slices of dark cutting muscle in air or oxygen. All of these treatments would inhibit mitochondrial respiration and allow myoglobin to remain oxygenated.

Cornforth and Egbert (1985) have shown that pre-rigor beef muscle homogenates will also remain red when blended with rotenone or pH 5.0 citrate buffer. Pre-rigor beef and dark cutting beef are both dark and have a high pH. However, several differences exist. Dark cutting beef muscle samples blended with water remained red when chilled on ice, where similarly treated pre-rigor samples turned dark (Cornforth and Egbert, 1985). Dark cutting muscle slices bloom when chilled to 3°C in air or when chilled to 14°C in an oxygen atmosphere, while similarly treated pre-rigor beef muscle slices remained dark (Cornforth et al., 1985). These color differences may be explained on the basis of relative mitochondrial respiration rate (Ashmore et al., 1972). Pre-rigor muscle samples have higher mitochondrial activity than dark cutting muscle samples, and remain dark at relatively lower temperatures or higher oxygen concentration.

Dark cutting beef muscle apparently has higher water binding capacity than pre-rigor muscle. A firm gel was formed when 50 mL of buffer is blended with 25g dark cutting muscle, but no gel was formed with 25g pre-rigor muscle. To prevent gel formation in the present study, 75 mL solution was blended with 25g dark cutting muscle to form a slurry. This led to some increase in redness and darkness of dark cutting muscle samples with time (Fig. 1, Table 1), due to settling of muscle homogenates in the Hunter color sample containers.

Table 1—Mean Hunter color 'L' values[a] for dark cutting rib muscle homogenates with time

Treatment	Time (min) 0[b]	10	20	30	40	50	60
Water at 20°C	47.1[c,d]	44.5	42.7	41.8	40.9	40.2	39.8
Buffer pH 5.0	50.5	47.7	46.3	45.1	44.1	42.8	42.1
Water at 3°C	48.2	46.9	46.6	46.4	46.5	46.3	46.1
Water and rotenone	49.4	48.1	47.1	46.3	45.5	44.5	43.9

[a] L = lightness; 100 = white, 0 = black
[b] 0 time = within 30 sec after blending
[c] Each value is the mean of four samples
[d] Least significant difference (LSD 0.01) = 2.68

Table 2—Mean Hunter color 'b' values[a] for dark cutting rib muscle homogenates with time

Treatment	Time (min) 0[b]	10	20	30	40	50	60
Water at 20°C	15.3[c,d]	14.3	13.5	12.1	11.4	10.8	10.6
Buffer pH 5.0	16.3	16.0	15.8	15.7	15.5	14.9	14.6
Water at 3°C	15.8	15.5	15.5	15.4	15.4	15.4	15.4
Water and rotenone	15.7	15.6	15.6	15.6	15.4	15.4	15.2

[a] b = yellow when positive, blue when negative.
[b] 0 time = within 30 sec after blending.
[c] Each value is the mean of four samples.
[d] LSD = 1.34

Table 3—pH of dark cutting rib muscle homogenates

Treatment	pH
Water at 20°C	6.46[a]
Buffer, pH 5.0	5.47[b]
Water at 3°C	6.44[a]
Water and rotenone	6.37[a]

[a,b] pH values with the same letter superscript are not significantly different ($p < 0.01$). LSD = 0.31.

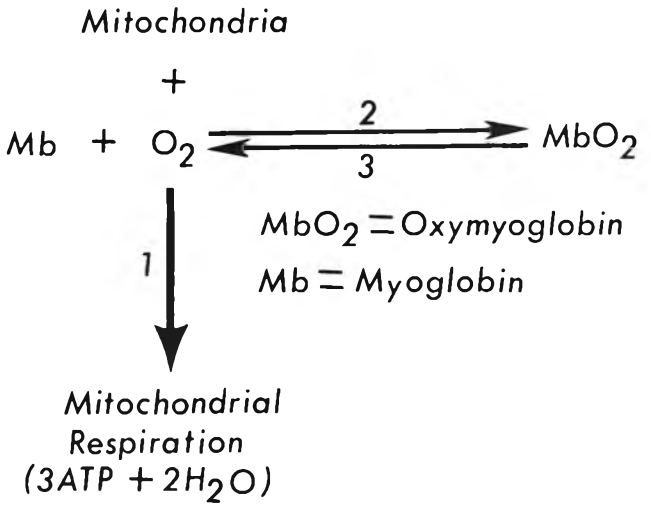

Fig. 3—Relationship of mitochondrial oxygen consumption to color of dark cutting meat. Myoglobin (Mb) is dark purple, and oxymyoglobin (MbO_2) is bright cherry red. Reaction 1 takes place preferential to reaction 2 unless mitochondrial respiration is inhibited by chilling, rotenone, or low pH. Bloom is enhanced if the sample is placed in an oxygen atmosphere, increasing the concentration of oxymyoglobin. Samples will turn darker when returned to lower oxygen tension (air) and higher temperature as mitochondrial respiration proceeds, and oxymyoglobin is deoxygenated via reaction 3.

Others have proposed that dark cutting or high pH muscle has higher water-holding capacity than normal post-rigor muscle, and therefore, less water is exuded from the muscle cells, resulting in a thinner layer of surface moisture. This thin layer of surface moisture reflects less incident light than normal post-rigor muscle, resulting in a darker perceived color (Orcutt et al., 1984; Lawrie, 1958; Forrest et al., 1975; Briskey and Kauffman, 1978; Romans and Ziegler, 1977; Fisher and Hamm, 1981; McDougall and Jones, 1981). However, treatment with rotenone did not appreciably affect the water-holding capacity of the dark cutting beef muscle slurries used in this study but did result in the development of bloom. These results indicate that mitochondrial respiration was probably responsible for the dark color of the dark cutting meat samples. Dark cutting meat is similar to pre-rigor meat, in that insufficient acid is produced during postmortem glycolysis to inactivate the mitochondria. As long as mitochondrial respiration continues, myoglobin will be deoxygenated and the muscle will remain dark (Fig. 3).

Currently, more practical methods to develop bloom in dark

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Table 4—Mean Hunter color 'L' values[a] for dark cutting rib muscle slices with time

Treatment	Time (min) 0[b]	10	20	30	60
Oxygen, 3°C to air, 20°C	26.7[c,d]	23.6	23.3	23.0	23.7
Oxygen, 3°C	27.5	27.0	27.6	27.4	27.7
Air, 3°C	26.0	26.0	26.2	26.1	26.6
Oxygen, 14°C	25.4	25.4	25.7	25.8	25.8
Air, 20°C	23.1	23.1	23.0	22.9	23.4

[a] L = lightness; 100 = white, 0 = black
[b] 0 time = 30 min after placing samples under treatment conditions
[c] Each value is the mean of four samples
[d] LSD = 4.47

Table 5—Mean Hunter color 'b' values[a] for dark cutting rib muscle slices with time

Treatment	Time (min) 0[b]	10	20	30	60
Oxygen, 3°C to air, 20°C	10.2[c,d]	7.6	6.6	6.8	6.4
Oxygen, 3°C	9.8	10.0	10.3	10.3	10.4
Air, 3°C	9.1	8.9	9.1	9.0	9.2
Oxygen, 14°C	8.5	9.0	8.8	8.7	9.4
Air, 20°C	6.6	6.8	6.6	6.8	6.8

[a] 'b' = yellow when positive, blue when negative
[b] 0 time = 30 min after placing samples under treatment conditions
[c] each value is the mean of four samples
[d] LSD = 1.40

Table 6—pH of dark cutting rib muscle slices

Treatment	pH
Oxygen, 3°C to air, 20°C	6.28
Oxygen, 3°C	6.25
Air, 3°C	6.28
Oxygen, 14°C	6.27
Air, 20°C	6.32

No significant difference ($p < 0.01$).

Algin/Calcium Gel as a Raw and Cooked Binder in Structured Beef Steaks

W. J. MEANS and G. R. SCHMIDT

ABSTRACT

Structured meat products which could bind raw as well as cooked would be superior to existing structured products. Therefore, the algin/calcium gel system was studied. Structured beef steaks were made using three levels of sodium alginate and three levels of $CaCO_3$. Three additional treatments were included as controls. Treatments were subjectively evaluated for six raw parameters: discoloration, color intensity, alginate pocket area, alginate pocket size, percentage fat and raw bind. Treatments were also subjectively evaluated for four cooked parameters: aroma, flavor, mouthfeel and cooked bind. The algin/calcium gel mechanism can be used to produce structured beef steaks which bind in both the raw and cooked state. Optimum ingredient levels were 0.8–1.2% sodium alginate, 0.144–0.216% $CaCO_3$ with 500 ppm sodium erythorbate.

INTRODUCTION

CURRENTLY, meat technologists use sausage technology to produce structured steaks resembling fresh, intact muscle cuts. These structured meat products are marketed either frozen or precooked to retain structural integrity. In existing products, cohesion between meat pieces is accomplished by formation of a heat set protein matrix after extraction of muscle proteins and subsequent heat treatment. Addition of sodium chloride and phosphate salts during mechanical manipulation aid in extraction of myofibrillar proteins involved in heat-set gelation (Pepper and Schmidt, 1975; Gillett et al., 1977; Siegel et al., 1978a; Theno et al., 1978; Solomon and Schmidt, 1980; Schmidt and Trout, 1982; Samejima et al., 1984). While the traditional myosin heat-set gelation mechanism can provide adequate binding of structured meat products in the cooked state (Siegel et al., 1978b; Solomon and Schmidt, 1980; Booren et al., 1981a; b; c; Wiebe and Schmidt, 1982), this system does not bind uncooked meat pieces. This paper addresses use of the algin/calcium reaction in the development of structured meat products which could be marketed in the raw, refrigerated state.

Although alginates are widely used in food systems as stabilizers and to modify the rheology of food sols, one of their more useful properties is gelation. Algin is different from other gel forming hydrocolloids because it forms chemically, rather than thermally, induced gels. Algin gels are formed by intermolecular association of polyvalent cations (except magnesium) with dimerically associated guluronic acid block regions of the polysaccharide molecule (Grant et al., 1973; Morris et al., 1977; Rees and Welsh, 1977; Morris et al., 1980). Calcium ions are superior to other polyvalent cations in their interaction with algin and are also the most commonly used source of cations in food systems (McDowell, 1966; Cottrell and Kovacs, 1980; Sanderson, 1981; Glicksman, 1982).

Several researchers have used alginate and the algin/calcium reaction to modify texture and influence structure of formed meat products. Ahmed et al. (1983) and Rockower et al. (1983) studied the effects of alginate addition on texture of minced fish patties with inconsistent results. Studies of sensory attributes of precooked, calcium alginate-coated pork patties (Wanstedt et al., 1981) indicated that a calcium alginate coating was useful in preventing warmed-over flavor of precooked pork. Alginates have also been incorporated into sausage products, for texture modification, and used as sausage casings (Abd El-Baki et al., 1981). However, this study mentioned no use of a calcium ion source. Allen et al. (1963) and Mountney and Winter (1961) reported a bitter flavor due to the relatively high calcium chloride levels which they used.

The objectives of this study were: (1) to evaluate the effectiveness of the algin/calcium gel as a binder in raw and cooked structured beef steaks; and (2) to evaluate the palatability of such steaks.

MATERIALS & METHODS

Experimental design

A balanced incomplete block design for subjective measurements according to plan S27 of Clatworthy (1973) was used for this experiment. This plan allowed for six panel members, or blocks, to evaluate six of the twelve treatments with two evaluators per each of three replicates. Treatments were arranged factorially with three levels of sodium alginate (Na-alginate; Manugel DMB, Kelco, Division of Merck and Company, Inc.) and three levels of calcium carbonate ($CaCO_3$; Gamma Sperse 80, Georgia Marble Co.), $3 \times 3 = 9$ treatments (See Table 1 for factor levels). Three additional treatments, no additives (NA), salt- and phosphate-structured beef steak (RS), and an intact muscle steak (IN) were included as controls, thus, giving a total of 12 treatments. This experiment was a balanced complete block design for objectively measured traits.

Variables

Treatments are shown in Table 1. Na-alginate and $CaCO_3$ levels were chosen based on the ideal algin:calcium ion ratio (2.5g algin:0.18g calcium) and $CaCO_3$ solubility (Anonymous, 1984). Fresh (2 days postmortem) USDA choice butt tenders (*M. Psoas major*) were used for the IN treatment whereas fresh ball tips (primarily *M.*

Table 1—Variables and experimental design

Treatment code[y]	Ingredients and level (%)[x]				
	NaAlg	$CaCO_3$	NaEryth	NaCl	STP
LL	0.4	0.072	0.05	--	--
LM	0.4	0.144	0.05	--	--
LH	0.4	0.216	0.05	--	--
ML	0.8	0.072	0.05	--	--
MM	0.8	0.144	0.05	--	--
MH	0.8	0.216	0.05	--	--
HL	1.2	0.072	0.05	--	--
HM	1.2	0.144	0.05	--	--
HH	1.2	0.216	0.05	--	--
NA[z]	--	--	--	--	--
RS	--	--	--	1.4	0.32
IN	--	--	--	--	--

[x] NaAlg = sodium alginate, NaEryth = sodium erythorbate, STP = sodium tripolyphosphate.

[y] First letter of treatment code refers to sodium alginate level (low, medium, high), second letter is calcium carbonate level.

[z] NA = no additives, RS = salt and phosphate structured beef steak, IN = intact muscle steak (*M. Psoas major*).

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Rectus femoris, M. Vastus lateralis) were used as the meat source for all structured steak treatments.

Product preparation

The IN treatment was prepared by trimming butt tenders so that only *Psoas major* muscle remained. These were then vacuum packaged (-8.4×10^4 N/m^2) and stored at 4°C until evaluated (2 days). Meat for all other treatments was ground once through a 1.905 cm × 4.763 cm kidney plate after removal of outside fat and connective tissue. The appropriate meat block for each treatment was randomly removed from this ground meat source. Alginate treatments were prepared by adding Na-alginate and sodium erythorbate (Heller Seasonings and Ingredients, Inc.) to the meat block and mixing in a Kitchen Aid mixer (model K45SS) on speed 2.5 for 30 sec. Alginate treatments were mixed for another 30 sec after addition of $CaCO_3$. The NA treatment was mixed for two 30 sec periods without additives. The salt- and phosphate-structured beef steak was formulated to an ionic strength of 0.33 with 1.4% sodium chloride (NaCl) and 0.32% sodium tripolyphosphate (STP; FMC Corp.) according to Trout (1984). Upon completion of mixing, ground treatments were stuffed into prestuck fibrous cellulose casings (10 cm flat width) with a hand stuffer. Casings were pulled to a constant tension (approximately 2.4×10^5 N/m^2) and clipped with a Tipper Tie clipper. Ground treatments were then vacuum packaged and stored at 4°C until evaluated (2 days).

Structured, stuffed beef rolls and the IN treatments were sliced into 2.0 cm thick steaks using a plexiglass template and a sharp knife. Steaks were placed onto a paper covered tray and allowed to bloom at 4°C for 1 hr. Raw characteristics were evaluated while steaks for cooked evaluation were being cooked on Farberware open hearth electric broilers (average temperature at steak surface = 160–170°C). Steaks were cooked for a constant time (AMSA, 1978) (11 min and 7 min per side) to a medium degree of doneness, as judged by standard color photographs. Cooked steaks were taken directly from the grill and placed on trays for immediate evaluation by panel members. Each panel member evaluated the same cooked treatments as raw treatments.

Subjective assessments

Nine prospective evaluators were trained during three 1-hr sessions. Samples representing the full range of treatments to be studied were evaluated independently before open discussion of each parameter. Six panelists were selected based on the consistency of their scores when compared to the entire group. Panelists evaluated treatments in a pass-through, cubicle type sensory evaluation room.

Raw product. Panel members evaluated raw treatments for six parameters. Six point descriptive scales were used for all parameters. Table 2 indicates the score description used for each raw parameter evaluated. Discoloration was defined as the brown muscle color associated with metmyoglobin and only that muscle tissue which did not resemble fresh red meat color was termed discolored. Color intensity was evaluated as the intensity of fresh red meat colors only, that is, discolored portions were excluded. Area of alginate pockets were scored as a percentage of total area. Alginate pockets were defined as small, irregular shaped, dark red pockets of alginate which were not distributed evenly throughout the product. The average size of alginate pockets was also evaluated (Table 2). Fat was evaluated as percentage of total steak area. Panelists were allowed use of a key which pictured a circle the size of a steak that was divided into 50, 25, 12.5 and 3% area pieces. The key also contained a 15 cm metric rule to aid in determination of alginate pocket size. Raw bind was evaluated by panelists who used a table fork to palpate each steak, thus determining particle to particle cohesion of the raw meat pieces. Raw bind was considered adequate if steaks could be handled sufficiently to be placed on a grill without falling apart. Samples of each raw treatment were frozen (−20°C) for pH and proximate analysis at a later time (5 months).

Cooked product. Six point descriptive scales were also used for evaluation of four cooked parameters (Table 3). Aroma and flavor were evaluated and compared to fresh grilled meat. Preliminary studies indicated that some alginate formulations gave a mealy or slippery mouthfeel. Therefore, mouthfeel was evaluated. Cooked bind was evaluated again using the fork palpation technique. However, panelists also cut cooked steaks with a serrated steak knife to aid in cooked bind evaluation. Cooked bind was considered adequate if the steak could be cut, using suitable knife and fork, without falling apart.

Objective assessments

Cook yield. Cook yield was expressed as (weight of cooked steak ÷ weight of raw steak) × 100 = % cook yield. One steak from each treatment (per replicate) was weighed before and after broiling to determine cook yield.

pH. Raw product was prepared by blending 1 part meat plus 4 parts deionized water in an Osterizer blender for two 15 sec periods. A Corning (model 125) pH meter in conjunction with a Corning combination electrode was used to measure hydrogen ion concentration of the meat slurry.

Proximate analysis. Moisture, fat and protein was determined for each treatment. Moisture was determined by drying homogenized, comminuted (chopped using a kitchen food processor) samples in a 60°C vacuum oven for 12 h. Extractable lipid was determined by pentane extraction of moisture-free samples using the Bailey-Walker apparatus. The Tecator system for Micro-Kjeldahl nitrogen determination was used for protein analysis. Percentage protein was calculated as % $N_2 \times 6.25$. All samples were prepared and run in duplicate.

Statistical analysis. Subjective parameters were analyzed as partially balanced incomplete block designs by analysis of variance (AOV) procedures as described by Cochran and Cox (1957) and Clatworthy (1973). Objective parameters were analyzed as complete block designs by AOV procedures (Snedecor and Cochran, 1976). The Statistical Package for the Social Sciences (SPSS) was used for all computer AOV procedures (Nie et al., 1975; Hull and Nie, 1981). The P-value plot procedure described by Schweder and Spjotvoll (1982) was used to decide on the significance level to be used for mean separation tests of both subjective and objective measurements. Orthogonal contrasts were used to determine P-values used for P-value plots and to analyze the controls grouped separate from the factorially arranged treatments (Snedecor and Cochran, 1976). This allowed analysis of the Na-alginate and $CaCO_3$ levels as factors in the factorial design. Least significant difference (LSD; Snedecor and Cochran, 1976) was used for all mean separation using an $\alpha = 0.10 \div T_o$ (where T_o = number of true null hypothesis, as derived from P-value plots). This method gives a protected LSD where the overall α level is equal to 0.10. Significance levels for all other comparisons will be given in results

Table 2—Score descriptions for subjective evaluation of raw steaks

	Raw parameters		
	% Discoloration	Color intensity	% alginate pockets-area
Score	*Score description*	*Score description*	*Score description*
6	No surface discoloration	Light cherry red	Devoid of pockets
5	<20% discoloration	Cherry red	Very few visible pockets (<1% area)
4	20-40% discoloration	Red	Slightly moderate no. of pockets (<2% area)
3	40-60% discoloration	Dark red	Moderate no. of pockets (<3% area)
2	60-80% discoloration	Very dark red, light purple	Numerous no. of pockets (3-5% area)
1	>80% discoloration	Dark purple	Many large pockets (>5% area)
	Alginate pockets-size	% fat	Raw bind
Score	*Score description*	*Score description*	*Score description*
6	0×0mm size, devoid of pockets	No visible fat	Very strong bind, comparable to intact muscle
5	1×1mm size	<10% fat	Strong bind
4	2×2mm size	10-20% fat	Moderately strong bind
3	3×3mm size	20-30% fat	Slightly weak bind
2	4×4mm size	30-40% fat	Weak bind
1	5×5mm size, or >	>40% fat	Virtually no bind

Table 3—Score descriptions for subjective evaluation of cooked steaks

	Cooked parameters			
	Aroma	Flavor	Mouthfeel	Cooked bind
Score	*Score description*	*Score description*	*Score description*	*Score description*
6	Fresh grilled meat aroma, no off odor(s)	Natural grilled meat flavor, no off flavor(s)	Same as intact muscle	Very strong bind, comparable to intact muscle
5	Very slight off odor(s)	Very slight off flavor(s)	Very slight mealy/slippery feel	Strong bind
4	Slight off odor(s)	Slight off flavor(s)	Slight mealy/slippery feel	Moderately strong bind
3	Moderate off odor(s)	Moderate off flavor(s)	Moderate mealy/slippery feel	Slightly weak bind
2	Strong off odor(s)	Strong off flavor(s)	Strong mealy/slippery feel	Weak bind
1	Very strong off odor(s)	Very strong off flavor(s)	Very strong mealy/slippery feel	Virtually no bind

and discussion. Reported results are the best linear estimates of treatment means.

RESULTS & DISCUSSION

Raw parameters

All treatments scored equal for percentage discoloration of raw steaks except RS (Table 4), which was significantly more discolored. The fact that NaCl increases the rate of oxidation of red meat pigments, oxyhemoglobin and oxymyoglobin, and/or hemoglobin and myoglobin to brown methemoglobin and metmyoglobin, respectively, has been known for many years (Coleman, 1951; Grant, 1956). More recent literature supports the effect of NaCl on product discoloration found in this study (Ockerman and Organisciak, 1979; Huffman, 1980; Booren et al., 1981b; Chastain et al., 1982).

In beef products where NaCl was added product discoloration increased as mixing time (Booren et al., 1981b), frozen storage time (Ockerman and Organisciak, 1979; Chastain et al., 1982), and refrigerated storage time (Ockerman and Organisciak, 1979) increased. The RS treatment had a darker color intensity than some factorial treatments when compared individually (Table 4). When the three controls (NA, RS, IN) were grouped and compared to the grouped factorial treatments, controls had significantly ($P<0.001$) darker color scores. The addition of sodium erythorbate to factorial treatments is probably responsible for less discoloration and lighter red color of these raw treatments. The protective effect of asorbic acid, an isomer of erythorbate, on fresh meat color has been reported previously (Rickert et al., 1957; Hood, 1975; Huffman, 1980).

As expected, control treatments had less than 1% of the surface area as alginate pockets (Fig. 1). Na-alginate level was a significant ($P<0.001$) factor with percentage alginate pockets and alginate pocket size increasing as Na-alginate level increased. Control treatments were determined to have small sized ($<1 \times 1$ mm^2) or no alginate pockets (Table 4) with other treatments being variable. The worst treatments for alginate pockets had 2–3% of the surface area (treatment HL) (% Na-alginate-% $CaCO_3$) as alginate pockets and pockets of size 2×2 to 3×3 mm^2 (treatment HM). These small dark red pockets may be undesirable to discriminating consumers. Alginate pockets are due to incomplete dispersion and hydration of Na-alginate during mixing of product. In commercial manufacture, however, alginate pockets may be avoided by controlled and careful addition of Na-alginate during mixing. There were no significant differences in perceived fat level among treatments (Table 4).

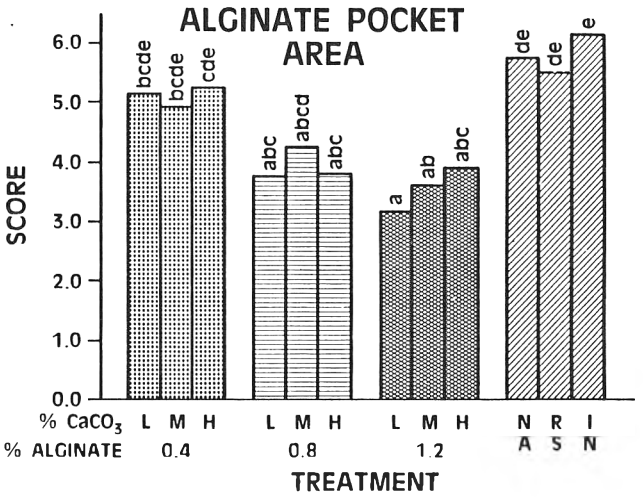

Fig. 1—Panel scores for alginate pocket area as percentage of total steak area. L. = 0.072, M = 0.144 and H = 0.216% $CaCO_3$. NA = no additives, RS = salt and phosphate structured beef steak, IN = intact muscle steak. Bars with different letters are different overall α = 0.10 for protected LSD.

Table 4—Panel evaluation results for several raw and cooked parameters

	Panel scores				
	Raw parameters			Cooked parameters	
Treatment code[w]	% discoloration[y]	color intensity	% fat	Alginate pocket size	aroma
LL	5.7^{bz}	5.8^{b}	4.6	5.3^{cdef}	5.1
LM	5.7^{b}	5.8^{b}	4.5	4.3^{abcd}	5.6
LH	5.8^{b}	5.6^{b}	4.9	5.1^{cdef}	4.9
ML	5.5^{b}	5.4^{b}	4.8	3.9^{abc}	5.1
MM	5.7^{b}	4.8^{a,b}	4.8	3.7^{ab}	5.2
MH	5.9^{b}	5.1^{a,b}	4.8	4.3^{abcd}	5.1
HL	5.4^{b}	5.5^{b}	4.6	3.9^{abc}	5.1
HM	5.3^{b}	5.8^{b}	4.8	3.3^{a}	4.6
HH	6.1^{b}	5.9^{b}	4.5	4.8^{bcde}	4.9
NA[x]	4.5^{b}	5.1^{a,b}	4.8	5.9^{ef}	5.1
RS	2.1^{a}	3.1^{a}	4.2	5.6^{def}	5.1
IN	6.1^{b}	4.6^{a,b}	5.2	6.1^{f}	5.6

[w] First letter of treatment code refers to sodium alginate level (low, medium, high); second letter is calcium carbonate level

[x] NA = no additives, RS = salt and phosphate structured beef steak, IN = intact muscle steak.

[y] Numbers in same column with different superscripts are significantly different (overall α = 0.10 for protected LSD).

[z] Score of 1.0 = > 80% discoloration, 6.0 = no surface discoloration; score of 1.0 = dark purple, 6.0 = light cherry red for color intensity; score of 1.0 = > 40% fat, 6.0 = no visible fat; score of 1.0 = very strong off odor(s), 6.0 = fresh meat grilled aroma, no off odor(s).

The IN treatment was not different from MM, MH and HH treatments for raw bind (Fig. 2). Thus, the algin/calcium gel mechanism is an effective binder of raw meat pieces. The lowest Na-alginate level (0.4%) did not bind as well as IN, regardless of $CaCO_3$ level. These data suggest that threshold levels of Na-alginate and $CaCO_3$ exist and that the $CaCO_3$ level necessary for adequate raw bind was dependent on Na-alginate level.

Cooked parameters

Panelists detected no differences in cooked aroma (Table 4), indicating that none of the added ingredients contributed detectable off odors. Although two factorial treatments (ML, HM) were different from NA and IN treatments for flavor, no treatments were different from the RS treatment (Fig. 3). When analyzed factorially, there were significant Na-alginate level ($P=0.003$) and $CaCO_3$ level ($P=0.043$) effects on flavor. Panelists detected an increase in off flavors as Na-alginate

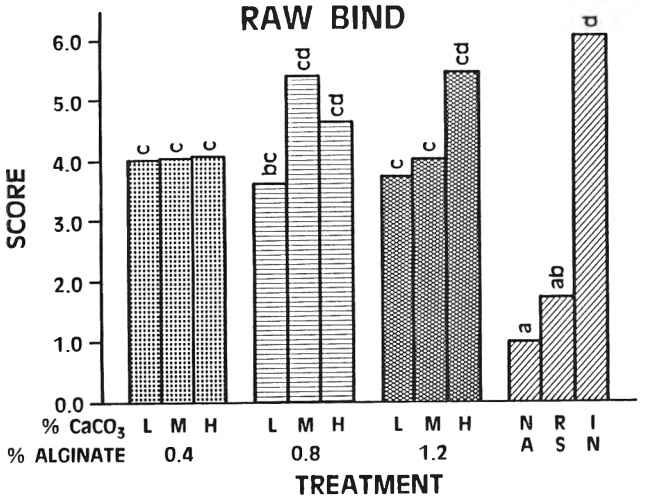

Fig. 2—Panel scores for raw bind. L = 0.072, M = 0.144 and H = 0.216% $CaCO_3$. NA = no additives, RS = salt and phosphate structured beef steak, IN = intact muscle steak. Bars with different letters are different overall α = 0.10 for protected LSD.

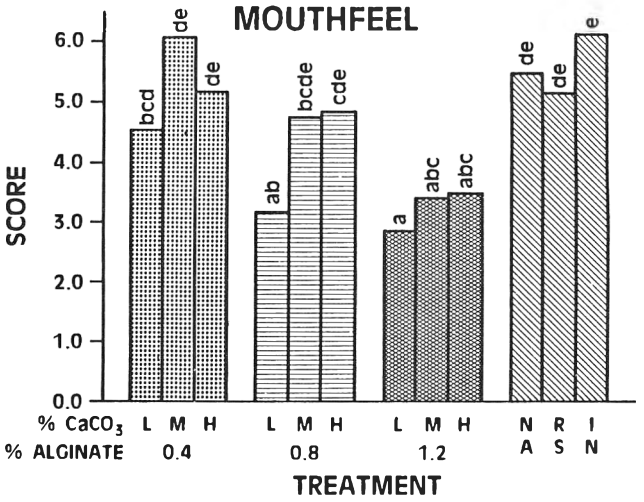

Fig. 4—Panel scores for mouthfeel. L = 0.072, M = 0.144, and H = 0.216% $CaCO_3$. NA = no additives, RS = salt and phosphate structured beef steak, IN = intact muscle steak. Bars with different letters are different overall α = 0.10 for protected LSD.

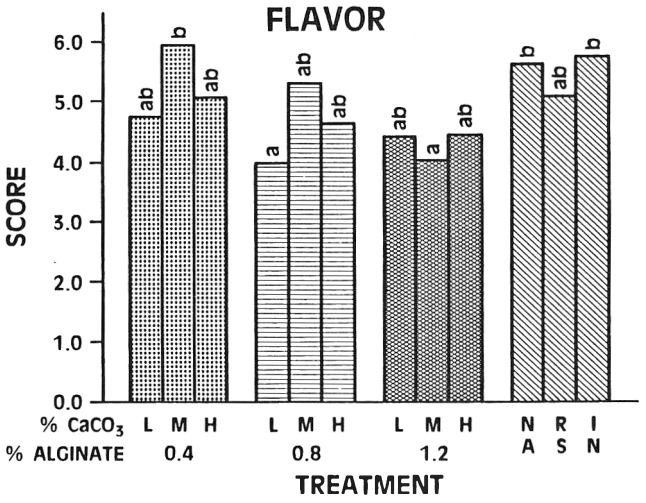

Fig. 3—Panel scores for flavor. L = 0.072, M = 0.144, and H = 0.216% $CaCO_3$. NA = no additives, RS = salt and phosphate structured beef steak, IN = intact muscle steak. Bars with different letters are different overall α = 0.10 for protected LSD.

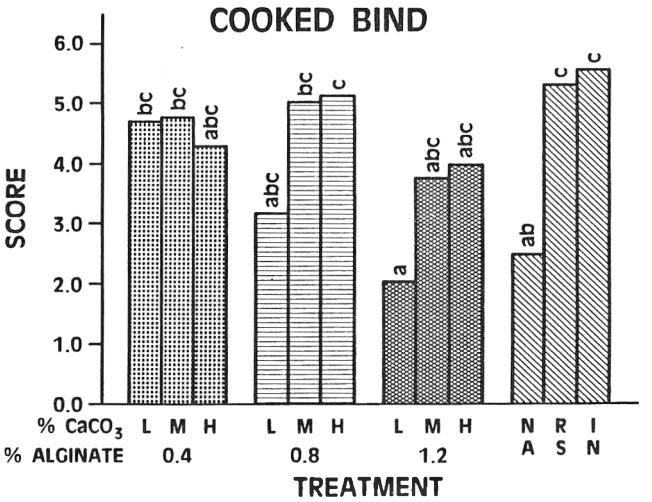

Fig. 5—Panel scores for cooked bind. L = 0.072, M = 0.144, and H = 0.216% $CaCO_3$. NA = no additives, RS = salt and phosphate structured beef steak, IN = intact muscle steak. Bars with different letters are different overall α = 0.10 for protected LSD.

levels increased. However, more off flavors were detected with the lowest $CaCO_3$ level (0.072) versus medium (0.144) or high (0.216) levels. Sodium alginate which has not yet reacted with calcium ions was responsible for detected off flavors.

Mouthfeel has not been a problem in salt and phosphate type products and, in this study, neither RS or NA differed from IN (Fig. 4). Panel evaluation scores for mouthfeel were highly variable for the factorial treatments (Fig. 4). Several factorial treatments (LM, LH, MM, MH) were not different from IN, indicating that higher $CaCO_3$ levels (0.144 and 0.216%) were necessary to deter a slippery or mealy mouthfeel and that high Na-alginate levels were detrimental to mouthfeel. These differences are supported by significant ($P<0.001$) Na-alginate and $CaCO_3$ level effects.

Several treatments received cooked binding scores which were not different from control treatments, RS and IN (Fig. 5). Previous reports also indicate that structured beef products can be made, using salt and phosphate, which possess adequate cooked binding characteristics (Pepper and Schmidt, 1975; Ockerman and Organisciak, 1979; Booren et al., 1981a; b,c; Wiebe and Schmidt, 1982). Only one noncontrol treatment (MH) was significantly different from NA. Although actual cooked binding scores ranged from 3.11 to 5.56, the mean separation technique did not detect differences for this trait due to relatively large variation of panel scores. There were significant Na-alginate ($P=0.006$) and $CaCo_3$ ($P=0.024$) level effects for cooked binding, with high Na-alginate levels (1.2%) and low $CaCO_3$ levels (0.072%) being detrimental to cooked binding.

Cook yield

There were no significant differences detected for cook yield. Cook yields ranged from 75.6 to 82.3%.

Proximate analysis

There were no detectable differences for percentage extracted lipid, which is in agreement with panel results. There were only minor differences in moisture and protein content between treatments. These are shown in Table 5, and are probably due to nonhomogeneity of the initial meat block.

Table 5—Proximate analysis and pH values

Treatment code[v]	Moisture (%)[x]	Fat %[y]	Protein %[z]	pH
LL	71.1^{bc}	5.3	20.9^{ab}	5.80^{c}
LM	70.8^{bc}	5.5	21.0^{ab}	5.96^{de}
LH	70.4^{abc}	5.9	20.9^{ab}	6.02^{ef}
ML	70.7^{abc}	5.5	20.8^{ab}	5.82^{cd}
MM	70.4^{abc}	5.5	21.2^{b}	6.03^{ef}
MH	70.7^{abc}	4.9	21.2^{b}	6.03^{ef}
HL	70.2^{ab}	5.8	21.0^{ab}	5.90^{cde}
HM	69.9^{ab}	6.0	20.7^{ab}	6.03^{ef}
HH	70.4^{abc}	5.6	20.6^{ab}	6.13^{f}
NA[w]	71.1^{bc}	5.2	21.4^{b}	5.45^{a}
RS	69.1^{a}	5.7	20.9^{ab}	5.85^{cd}
IN	71.9^{c}	6.0	19.8^{a}	5.63^{b}

[v] First letter of treatment code refers to sodium alginate level (low, medium, high); second letter is calcium carbonate level

[w] NA = no additives, RS = salt and phosphate structured beef steak, IN = intact muscle steak

[x] Numbers in same column with different superscripts are significantly different (overall α = 0.05 for protected LSD).

[y] Lipid extractable using pentane and Bailey-Walker method.

[z] Kjeldahl nitrogen × 6.25.

pH

The average pH of all factorial treatments (5.97) was significantly ($P<0.05$) higher than that of the NA treatment (5.45). Since high pH meat (pH = 5.8–6.0) is known to spoil faster and with different microflora than normal pH meat (Egan, 1984), a method of maintaining a more normal product pH (pH~5.5) may be useful. Addition of various food grade acidulants could prove essential to maintaining an acceptable shelf life of Na-alginate/$CaCO_3$ structured beef steaks. Further work in this area is warranted.

SUMMARY

THE ALGIN/CALCIUM gel mechanism can be used to produce structured beef steaks which bind not only in the cooked state, but also in the raw, refrigerated state. These results indicate that 0.8–1.2% Na-alginate and 0.144–0.216% $CaCO_3$ with sodium erythrobate (500 ppm) are optimal ingredient levels in this model system. Further research is necessary to determine if this binding system is feasible on a commercial scale. Research efforts should also be directed toward microbial stability of such products and to elucidate any algin-protein interactions and thus increase cooked bind.

The algin/calcium gel mechanism allows structured meat products to be marketed at the retail meat case with other fresh meats. This technology would also allow structured beef to be marketed to the hotel, restaurant and institutional industries through existing fresh meat channels. Because freezing of this product is not required, energy savings can be realized during marketing and final cooking from the raw versus frozen state. The algin/calcium binding mechanism could also prove useful in development of pre-cooked, microwaveable structured beef steaks. This technology may represent a new generation of formed meat products which are void of some problems associated with salt and phosphate products.

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*EFFECT OF LOW-CA-REQUIRING CAF ON MYOFIBRILS. . .*From page 32

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*COLOR OF DARK CUTTING BEEF MUSCLE. . .*From page 59

cutting beef are being studied. Injecting dark cutting beef longissimus muscle with 1.0M food grade lactic acid successfully lowered pH to 5.77, but the muscle remained dark. There were additional problems of pigment oxidation and brown color development on some portions of the meat surface. The injected acid apparently did not sufficiently lower pH within muscle fibers, since dark cutting muscle homogenates with pH adjusted to 5.47 with 0.2M citrate did bloom. A slow freeze-thaw should also produce bloom by rupturing mitochondrial membranes, but this did not occur when dark cutting meat was so treated, apparently because freeze-damaged mitochondria retain sufficient respiratory capability to keep the muscle dark.

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Utilization of Glucose Oxidase for Extending the Shelf-Life of Fish

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ABSTRACT

The preservative benefits of the enzymic glucose oxidase/catalase system were measured in refrigerated, fresh, whole winter flounder (*Pseudopleuronectes americanus* Waldbaum) and winter flounder fillets. The enzyme system was applied as a dip, as an ice and immobilized in algin blankets. Advantages were an extended period of sensory acceptance and delayed onset of putrefactive odors, 21 days vs 15 days for controls. The pattern of endogenous hypoxanthine accumulation and decline was unaffected, but parameters thought to measure proteolytic activity were modified: creatinine turnover was slowed and ammonia generation was retarded.

INTRODUCTION

THE RECENT TREND of seafood consumption in the U.S. has been toward various fresh forms of fish (Steinberg, 1981). Since the U.S. fishing industry is the main supplier for the fresh seafood market (Campbell, 1982; Steinberg, 1981), careful attention to consumer demand for high quality commodities is required if the industry is to continue to expand (Campbell, 1982). With the high demand for fresh fish far from local fishing ports, the need for long term fresh fish preservation techniques, which begin from the point of harvest, maintain quality and promote sales, must continue to be addressed.

The assurance of fresh fish quality encompasses the degree of freshness and any deterioration throughout the period of harvesting, storage, processing, distribution, and sale (Connell, 1975). As early as 1969, the possible inadequacies of ice preservation for the distribution of fresh fish in the expanding marketplace was noted and the need for its replacement or supplementation was affirmed (Holston and Slavin, 1969). However, any new methodology would not only have to extend shelf-life of fish in a safe manner and result in a quality product, but it must fit easily into existing seafood industrial technology.

The discovery of an antimicrobial agent in the growth products from molds, led to an active search for other antibiotics during the 1940s. One of the products of these efforts was a compound called notatin (or penicillin B), isolated from the culture broth of *Penicillium notatum* (Coulthard et al., 1942; Kocholaty, 1943; Van Bruggen et al., 1943.). This compound turned out to be an enzyme, catalase-free glucose oxidase (GOX), which was thought to exert antimicrobial effects due to production of hydrogen peroxide (Scott, 1975). This enzyme has been employed in the U.S. food industry since the 1950s, not so much for its bactericidal effects but for its desugaring and antioxidant capacities (Scott, 1975; Hasselberger, 1978; Whitaker, 1980).

The general reaction required for GOX to function in food systems was described by Rand and Hourigan (1975). Rand (1972) and Rand and Hourigan (1975) developed a process for the preservation of foods utilizing GOX via the enzymatic production of acid. The combination of the "antibacterial" effect of the enzyme and acid production could prove to be an effective agent in the preservation of fresh fish. The purpose of this study was to evaluate the preservative capabilities of the enzyme on fish and to design and implement a unique GOX system for preservation of fresh fish which could be introduced into existing processing technology.

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METHODS & MATERIALS

Fish procurement and handling

Trawl-caught winter flounder (*Pseudopleuronectes americanus* Waldbaum; average weight, 106.32g; average length, 21.53cm) were supplied by local fishermen. Fish were received from day boats at time of landing (ca 6–7 p.m.). The whole fish were packed in rows, white side up, on ice in insulated chests and brought to the laboratory where they were stored packed at 2–4°C overnight. Early the next morning, whole fish and skinless fillets were treated with the enzyme dip, enzyme ice or enzyme algin blankets and held at 2–4°C.

Enzyme screening assay

A commercial enzyme preparation containing glucose oxidase (EC 1.1.3.4) and catalase (EC 1.11.1.6) was donated by Fermco Biochemics, Inc., Elk Grove Village, IL and used throughout this study. A modification of the disc assay for penicillin in milk (APHA, 1960) was utilized to evaluate enzyme levels (0.1–0.9, 1.0–10.0, 50, 100 Units (U)/mL) and glucose concentrations (0–10, 15, 20%). Enzyme units used in this study were those defined and assayed by Fermco Biochemics with 1.0 mL containing 1500 units of glucose oxidase activity. A combination of 1 unit GOX (with catalase) and 4.0% glucose effected optimum bacteriostases against *Bacillus subtilis* (Field, 1981) and was used throughout these trials.

Enzyme surface dip

An enzyme solution of 1 U/mL was prepared by adding 1.0 mL of GOX/CAT to 1499 mL of refrigerated (28°C) deionized, distilled water in a stainless steel receptacle. Immediately prior to dipping of fish, 60g of glucose were added to the enzyme solution and slowly stirred until dissolved. Fillets or whole fish were placed into this enzyme substrate (E/S) solution for 1 min with intermittent mixing. After dipping, the fillets or whole fish were removed, drained, and individually packaged in labelled, sterile Whirlpak® bags (Cole-Parmer Instrument Co., Chicago, IL) or placed in stainless steel, covered trays for storage at 2–4°C. Controls were either dipped similarly in deionzed, distilled water or stored undipped. Packaging procedures and storage temperatures for controls were identical to those for the treated fish.

Enzyme ice

An E/S solution was prepared by dissolving 240g of glucose in 6L of deionized, distilled water and adding 4.0 mL of GOX/CAT, resulting in 1.0 U GOX/mL. After mixing, the E/S solution was frozen at −20°C in covered, plastic freezer containers. After freezing, the ice blocks were removed from the containers and crushed. Whole winter flounder were packed, white side up, in layers of the crushed E/S ice and stored at 2–4°C. Surface temperature of the fish was monitored and remained at 0–1°C. Controls were similarly packed in freshwater ice.

Enzyme/algin blankets

A 1.0% algin solution was prepared by boiling 3L of deionized, distilled water, adding 30g of Improved Kelmar® algin (Kelco Division of Merck and Co., Rathway, NJ), and stirring until dissolved. The resulting algin solution was divided into two 1500 mL aliquots, one containing 1 U GOX/mL using the GOX/CAT enzyme mixture

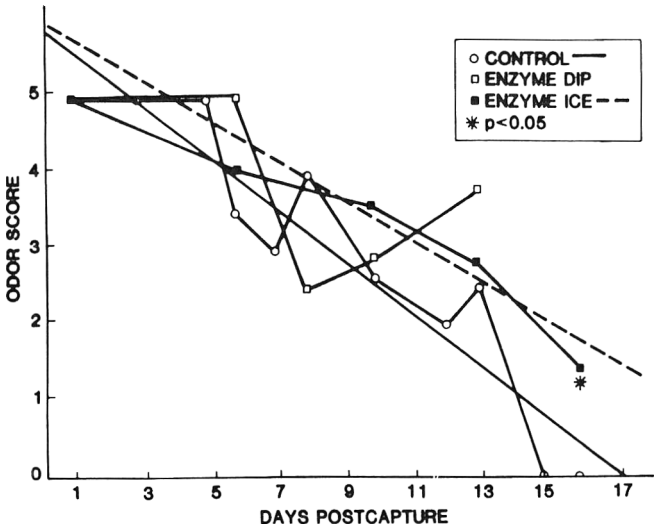

Fig. 1—Odor evaluation of fillets from fish stored whole.

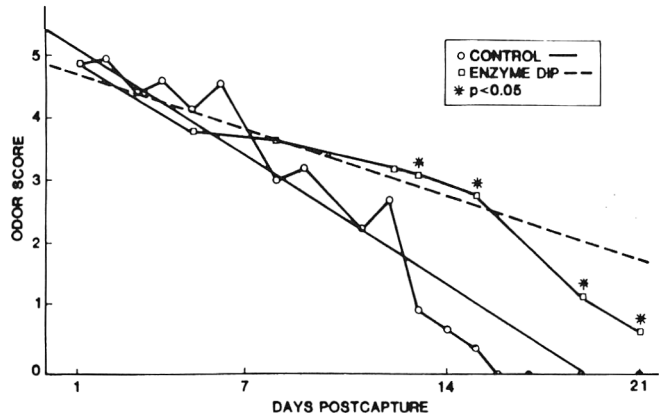

Fig. 2—Panel odor evaluation of refrigerated, enzyme-dipped winter flounder fillets compared to refrigerated controls.

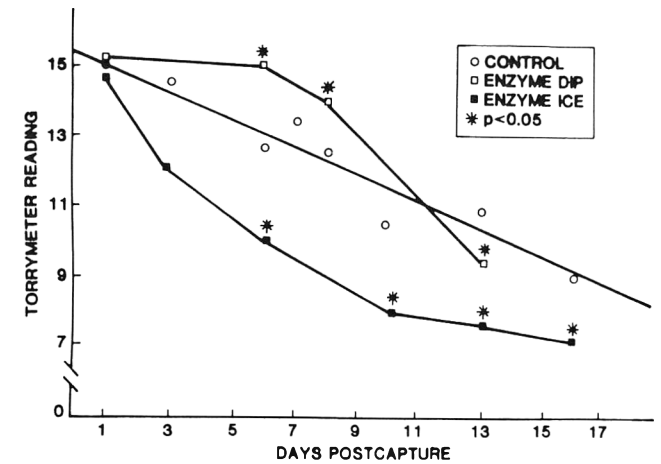

Fig. 3—Torrymeter readings at various days postcapture for enzyme treated, whole winter flounder.

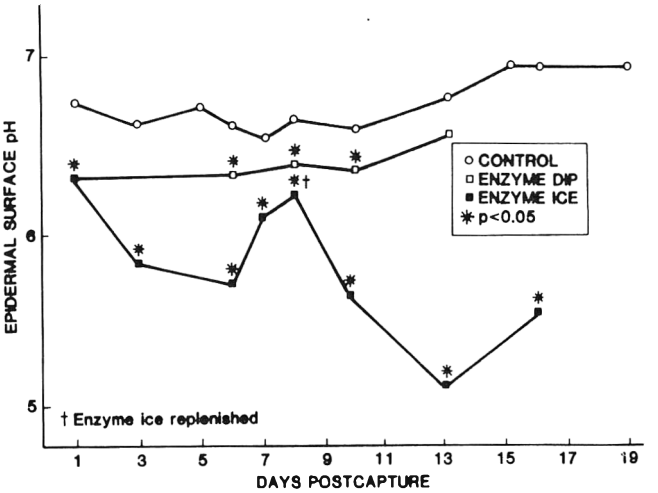

Fig. 4—Epidermal surface pH at various days postcapture for enzyme treated and control whole winter flounder.

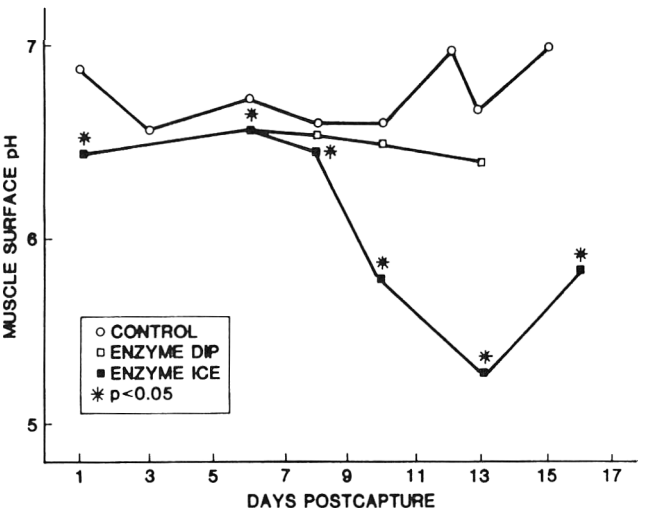

Fig. 5—Muscle surface pH of enzyme treated and control whole winter flounder at various days postcapture.

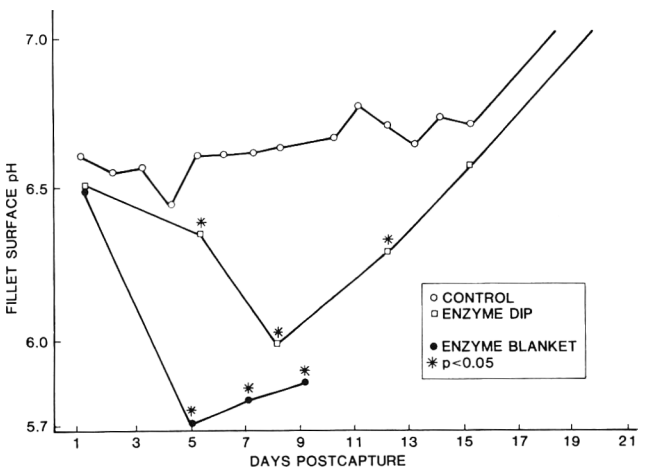

Fig. 6—Surface pH of enzyme treated and control winter flounder fillets during refrigerated storage.

and one for control algin blankets. A 350-mL aliquot of the enzyme-alginate mixture was poured into a 30 X 15 cm stainless steel tray. A 10.0% $CaCl_2$ spray was applied to partially set the gel. Additional 10.0% $CaCl_2$ was carefully poured at one end of the tray until the algin blanket was lifted from the tray bottom and was covered with the crosslinking agent as well. The blanket was allowed to set in the $CaCl_2$ for 1 min and then washed under cold, tap water for at least 2 min. After draining excess water by squeezing gently, the blankets were stored, covered, at 2–4°C until used.

Skinless fillets were dipped for 1 min with intermittent swirling in a 4.0% glucose solution. After draining, alternating layers of fillets

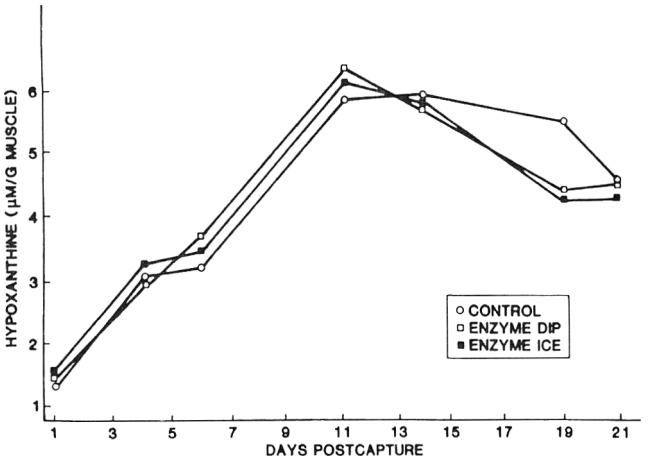

Fig. 7—Patterns hypoxanthine accumulation in enzyme treated and control winter flounder during 21 days storage.

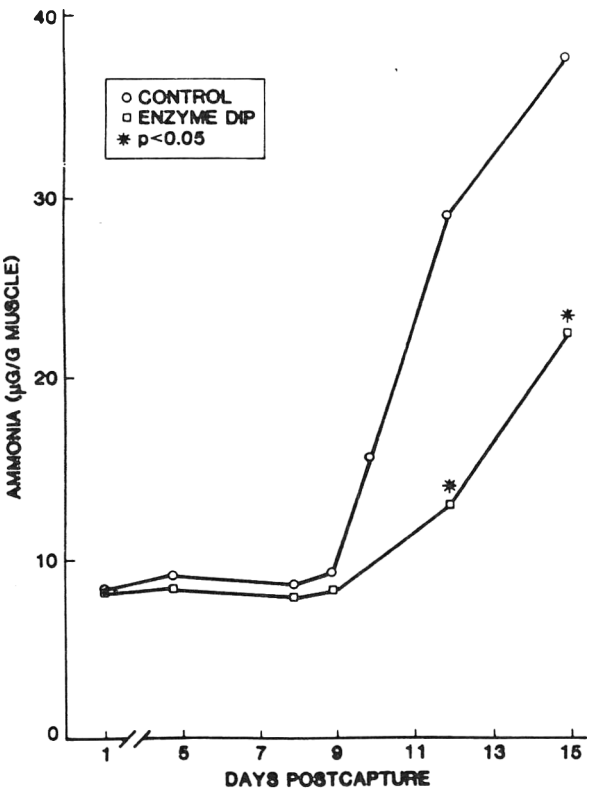

Fig. 8—Ammonia development in enzyme-dipped and control winter flounder fillets stored at 2–4°C.

and GOX/CAT algin blankets, beginning with a bottom enzyme-algin blanket and ending with an overlying enzyme-algin blanket, were placed in stainless steel trays and stored at 2–4°C. Control algin blankets, without enzyme, were similarly prepared and undipped control fillets similarly packaged and stored at 2–4°C.

Quality measurements–At predetermined days postcapture, duplicate or quadruplicate samples of both treated and control fish were taken for subjective and objective evaluation of quality. Subjective indices involved sensory evaluation of fillets stored as such or fillets from fish stored whole which were coded and presented to at least 6 members of a 16-member laboratory panel (Larmond, 1977). Raw sensory analysis based on odor was obtained using an evaluation sheet which identified possible perceived odors which accompany fish deterioration. The six descriptive categories were as follows: fresh or shellfish, no odor, fruity, sweet or acetic, slightly fishy, stale cabbage, and very fishy, ammoniacal or putrid. Panelists were asked to evaluate the fish fillets and check the category they felt most closely described the perceived odor. The odor choices were then assigned a value from 0–

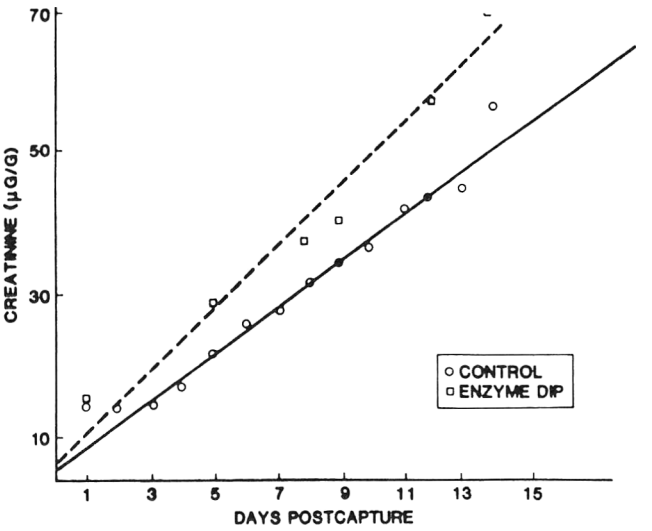

Fig. 9—Creatinine accumulation in enzyme treated and control winter flounder fillets stored at 2–4°C.

5 by the researchers: 5 being fresh or shellfish and 0 being very fishy or putrid. Dipped fillets were also evaluated for cooked flavor. Treated and control fillets were rinsed and individually packaged in coded, sterile Whirlpak® bags. The bags were placed in a boiling water bath for 3 min. Panelists were asked to evaluate the cooked fillets based on a 5-point hedonic scale, 5 the best and 1 the worst, and were invited to make additional comments.

In those trials where fish were stored whole, the GR Torrymeter (NOVA and Co., Manchester, MA) was used to estimate quality. Triplicate measurements were taken for each fish by moving the meter slightly in position (2 mm maximum) parallel to the lateral line.

When the fish were treated whole, the surface pH of both the epidermis and the excised muscle was measured using a flat-surface pH electrode (0–1 pH 2000, Owens-Illinois, Inc., Toledo, OH) and a Corning Model 12 pH meter. When the fish were treated as fillets, the fillet surface pH was measured.

Hypoxanthine, ammonia and creatinine

Treated and control fish samples, 20g, were extracted with 80 mL of cold, 1.0N perchloric acid by blending at high speed for 1 min in a Waring Blendor for measurement of hypoxanthine. The extracts were filtered through Whatman #2 paper and stored at 2–4°C until analysis. A similar extraction procedure was performed for ammonia analysis with 10g of fish extracted with 30 mL of acid. Hypoxanthine was determined by the procedure of Jahns and Rand (1977) as modified by Jacober (1981).

Ammonia was analyzed using an Orion Microprocessor Ionanalyzer 901 and Orion ammonia electrode. Using the anilate addition method as described by Orion (1979), 1.0 mL of perchloric acid extract was added to a 100 mL solution consisting of 99 mL of 0.4M NaOH and 1.0 mL of 100 ppm ammonia standard. Results were expressed as μg/g of fish muscle. All samples were tested in duplicate.

Creatinine was monitored by a modified procedure of Tietz (1976). A 10-g sample of fillet was extracted with 30 mL of a cold solution of equal parts of 0.33M sulfuric acid, 5.0% sodium tungstate and distilled water and blended at high speed for 1 min in a Waring Blendor. The extracts were filtered through Whatman #2 paper to remove protein. A 2.0 mL fraction of the clarified fish extract was added to 2.0 mL of deionized, distilled water, followed by addition of 1.0 mL of 0.04M picric acid and 1.0 mL of 0.75M NaOH, with mixing after each addition. Samples were incubated at room temperature for 15 min and the absorbance was measured at 500 nm with a Bausch and Lomb Spectronic 21 spectrophotometer. Results were calculated using a standard curve obtained from similarly treated, freshly prepared standards, 0–80 μg creatinine (Sigma Chemical Co., St. Louis, MO). All fish samples were tested in duplicate.

Data analysis

In each trial, treated and control fish were sampled in duplicate or quadruplicate depending on the quantity of fish available, the number of sampling days and the form of treatment. All measurements were performed at least in duplicate and therefore, for any given day, con-

trol and treated values reported represent the means of at least eight individual analyses. Where applicable, linear regression coefficients were calculated. Where statistical analysis was appropriate, the Student's t-test was performed.

RESULTS & DISCUSSION

EVALUATION of odor of fish in cold storage demonstrated that the laboratory panelists found the enzyme treatment elicited some favorable effects. The panel score for fillets excised from treated whole fish exhibited a trend toward improved odor which is shown in Fig. 1. However, statistical significance was not apparent until a very late stage of storage.

The panel score for treated fillets showed that the differences between enzyme-treated fish and controls were more pronounced, as demonstrated in Fig. 2. For the first 5 days of storage at 2–4°C, the fillets were all judged to have a sea fresh or no odor. The controls exhibited fruity odors by day 8, slightly fishy odors by day 11, stale cabbage odors by day 13 and very fishy, ammonia-like or putrid odors by day 15. In contrast, fruity odors persisted in treated fish until day 15, after which deterioration leading to slightly fishy, day 17, and stale cabbage, day 19, odors ensued. Only after about 21 days of storage did treated fish exhibit putrid and ammonia-like odors.

These relationships were statistically significant only after the first 2 wk of storage, a situation reflecting the large variation in sensitivities of panelists, individuals who shared a liking for and some experience with purchasing or experimenting with fish. In addition, the cultural backgrounds of the evaluators were varied. Some individuals, Orientals and Saudi Arabians for instance, seemed less critical of odors that were clearly undesirable to American panelists. However, since the Rhode Island community is welcoming growing numbers of such individuals, the laboratory panel, while not an "expert" one, may more closely resemble the ethnic mix of consumers in the Rhode Island area.

Among objective indices of quality assessment, the pattern of steady decline expected for the Torrymeter on whole, iced fish, was altered by the enzyme dip and ice treatments (Fig. 3). The enzyme dip resulted in a significant retardation, at least until day 9, of the usual loss in quality as measured by this instrument. However, the enzyme ice treatment resulted in an almost immediate, sustained and statistically significant decline in the dielectric properties of the whole fish. This appeared to be due to an excess acid generation by the enzyme ice and accumulation of the gluconic acid at the skin/muscle interface. Therefore, the Torrymeter readings were lower than expected for fresh, whole winter flounder when stored with the ice preservative.

These phenomena may be explained somewhat by observed changes in surface pH. For dipped, whole winter flounder, an immediate, moderate, sustained and statistically significant decrease in epidermal surface pH, as compared to the control was encountered (Fig. 4). However, the extreme decline in epidermal surface pH exhibited by fish packed in enzyme-ice contributed to an erosion of the skin which was apparent as early as day 7. At this time and throughout the remaining storage period, the skin could easily be rubbed by hand from the fish, leaving behind exposed muscle and naked fin rays. The great potential of the GOX/CAT system for generating acid was apparent when the pH of the crystal clear, collected melt water was found to be 3.55 on day 5. Therefore, excess acid generation and the accompanying undesirable epidermal changes of fish when stored in the GOX ice must be controlled. This is an area now being studied.

Measurements of muscle surface pH for whole flounder in refrigerated storage followed a similar trend, as seen in Fig. 5. For enzyme-dipped fish, a small decline was noted, suggesting that not much of the generated acid penetrated the skin. Enzyme-iced fish exhibited an extreme drop in pH after day 7 which was consistent with the described problem of eroding skin.

Table 1—Taste evaluation of cooked, enzyme-dipped and control winter flounder fillets at 5, 7, and 9 days of refrigerated storage

Day postcapture	Taste score/Comments (X ± 1 SD)			
5	Treated		Controls	
	4.3 ± 0.47	More bland; tougher texture; a little better.	4.0 ± 0.82	More fishy tasting; stronger taste; good texture
7	4.5 ± 0.5	Good, firm texture; more fresh fish flavor; no odor.	3.17 ± 0.9	Mushy; stronger fish flavor
9	4.0 ± 0.0	Slightly fishy taste, but adds character; texture very good; color very good; far better all around.	1.5 ± 0.5	Definitely spoiled taste; strong ammonia/fishy odor; fishy odor in bag.

The surface pH pattern of treated fillets is presented in Fig. 6. The pH of dipped fillets was decreased significantly with respect to controls for at least 12 days postcapture. Fillets layered in enzyme-alginate blankets exhibited even lower surface pH values, an observation that may be consistent with a reported shift in pH optimum from 5.5 to 6.5 for immobilized GOX (Bjorck and Rosen, 1976).

Hypoxanthine levels appear to be largely unaffected by enzyme treatment (Fig. 7) and exhibited the expected increase and decrease pattern (Jacober and Rand, 1982). This finding is consistent with the observation that freshness, which is an expression of endogenous enzymatic activity, may not be affected by GOX/CAT treatment. However, the odor scores obtained on the control fish are consistent with the day 11 hypoxanthine peak.

Ammonia levels were significantly reduced in treated fish as demonstrated in Fig. 8. Ammonia increased rapidly as the control entered the spoilage phase, a process that was retarded in the enzyme-treated fish. In light of the clear differences in ammonia development in treated versus control fish after day 9, this parameter may be a better measurement for incipient spoilage detection than the diamine accumulation criteria proposed by Jahns and Rand (1977). Levels of the cyclic anhydride of creatine, creatinine, have been found to gradually increase with refrigerated storage of fresh fish (Field, 1981). A trend of creatinine accumulation in treated fish was noted in these trials as shown in Fig. 9. These increased levels may correlate with a decreased turnover of this compound by spoilage organisms during low temperature storage. The observed accumulation could occur because trimethylamine (TMA) may be synthesized from creatine, the precursor of creatinine (Fields et al., 1968). In addition, psychrophillic microorganisms may be elaborating creatinine-degrading enzymes during early stages of spoilage. If the metabolism of putrefactive organisms were slowed, it is conceivable that creatinine levels could be higher in enzyme-treated fish. Lastly, cross-linking of protein brought about by decreased surface pH and water binding effected by gluconolactone (Anon., 1980) might tend to cause the retention of some water solubles such as creatinine, which ordinarily would leach out of the fillet and into the drip.

Finally, Table 1 illustrates the effect that the GOX preservative had on the flavor acceptance of cooked fillets. A taste panel of six individuals was utilized to evaluate the taste of cooked fillets stored for 5, 7 and 9 days at 2–4°C. The results presented in Table 1 indicate the superior quality of enzyme-dipped fillets. On day 5, panelists "liked very much" or "liked" both treated and untreated fillets, though fillets were considered by some respondents to be slightly tough. By day 7, no deterioration in acceptability was noted for treated fillets, but

control fillets were judged to be of neutral desirability. Interestingly, the firmer texture of treated fillets alluded to in day 5 evaluations was judged at day 7 to be a desirable characteristic in light of the softening controls. At day 9, the contrast between treated and control fillets was most clear; the treated fillet was "liked," while the untreated fillet was either "disliked" or "disliked very much." Therefore, this treatment had no adverse effects on the overall taste acceptability of cooked flounder fillets.

Parameters measured in these trials suggest that the GOX/CAT system described elicited favorable preservative benefits and delayed the onset of spoilage as reflected by ammonia, pH and sensory measurements. Benefits included a 6-day or 50% extended period of sensory acceptance for dipped fillets, where fishy odors were not detected in the treated fillets until day 17 and putrefactive odors until day 21 versus 11 and 15 days, respectively, for the controls. Furthermore, creatinine turnover was slowed and ammonia generation was retarded. There was improved Torrymeter readings for 8 days and reduced epidermal, muscle and fillet surface pH values for 12 days. It would appear that these benefits resulted from a combination of factors. The generation of acid as gluconic acid would serve to reduce pH which may be inhibitory (Lamanna et al., 1973) at least temporarily, to microorganisms acclimated to the neutral or slightly alkaline marine environment. In addition, the possibility that microbes, other than those traditional psychrotrophs, could be selected by the acidification process should be recognized. For instance, it is possible that members of the *Lactobacillaceae,* which are dominant in microbial succession in fermented foods and which cause a decline in proteolytic types, are synergists in this enzymatic preservation process since certain Lactobacilli secrete antimicrobial substances that are active at pH 3.5–6.0 (Genske and Branen, 1973). As Silliker and Wolfe (1980) note, the sensory changes in meat concomitant with growth of lactic acid bacteria are less noticeable than those changes detected when Gram-negative putrefactive forms dominate. The generation of hydrogen peroxide may inhibit the growth of psychrotrophic spoilage organisms, especially *Pseudomonas* spp., which are reported to be extremely sensitive to this chemical (Genske and Branen, 1973). However, the presence of catalase in the system should minimize the effect of hydrogen peroxide.

Other possible bacteriostatic products could include gluconic acid itself, a metal complexing agent (Wiseman, 1975) and gluconolactone which has been reported to be useful as a binding agent for water and metal ions (Anon., 1980). This water-binding capacity may be reflected in decreased weight loss noted in treated fillets measured in one trial (Field, 1981). Furthermore, Barua and Scheleff (1980) have reported that the growth of Pseudomonads was suppressed by glucose added to nutrient broth, a phenomenon attributed in part to lowered pH. Another factor, of undetermined significance, would be an altered gaseous microenvironment where oxygen has been slowly depleted in the interstices of the epidermal or muscle surface by the enzyme action.

This proposed scheme is supported by results described in a Japanese patent (Fukazawa, 1980), where GOX or "substances possessing glucose oxidase activity" were shown to be effective in preserving proteinaceous foods, including minced chicken, tofu, and Kamaboko, a type of fish gel. This paper, however, represents the first such report of an enzymatic process to preserve fresh fish. Applications to other fish species, both fatty and lean, and the control of acid generation in the enzyme ice are now being tested in our laboratory.

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Partial Purification and Characterization of Cathepsin D-Like and B-Like Acid Proteases from Surf Clam Viscera

H.C. CHEN and R.R. ZALL

ABSTRACT

Different proteases were isolated and purified from viscera of surf clam, *Spisula solidissima*. These proteases were similar to cathepsins D and B, with molecular weights of ~36,700 and ~17,400 daltons, respectively. Optimum activity of enzymes towards hemoglobin and casein occurred from pH 2.5–3.0 and temperature 44–46°C. Cathepsin D-like protease, a carboxyl protease, was insensitive to most protease inactivators, but extremely sensitive to pepstatin. Cathepsin B-like protease, a thiol protease, was activated by thiol-reducing agents and metal chelators, but was sensitive to many reagents such as iodoacetamide, Tosyl-phenylalanine chloromethyl ketone, Tosyl-lysine chloromethyl ketone, phenylmethanesulphonyl fluoride, leupeptin and heavy metals.

INTRODUCTION

MUCH INFORMATION is currently available for proteases found terrestrially in plants, microbes and higher animals. However, the properties and functions of proteases which may be found in clams are still relatively unknown. Reid and Rauchert (1970) demonstrated that tryptic and chymotryptic endopeptidases, carboxypeptidases A and B, and leucine aminopeptidase were present in the gastric juice and diverticula extract of *Chlamys hericius*. Although these investigations concentrated on alkaline endopeptidases from bivalves, it was realized that the greater portion of protein digestion occurred intracellularly at acid pH levels in the digestive diverticula. Recently, D-like and B-like catheptic activities were found in *Tresus capax* (Reid and Rauchert, 1976) and two copper-activated acid proteases were isolated and crystallized from their zymogen forms of a sea mussel, *Mytilus galloprovincialis* (Iordachescu et al., 1978). Aikawa and Aikawa (1982) investigated the distribution of lysosomal acid proteases in the tissues relating to various functions other than the digestion of nutrients. In 12 mulluscans surveyed they found that cathepsin D-type proteases with an optimum pH around 3 were predominant in various tissues, such as adductor muscle, foot, gill, mantle and midgut gland.

The surf clam, *Spisula solidissima*, is one of the relatively new seafood resources of the Atlantic coast shellfish processing industry. Appreciable amounts of wastes, including clam wash water, bellies and shells are generated along with the clam meat in the processing line. The clam belly which constitutes from 7 to 25% of the total meat is currently underutilized and causes a disposal problem. This study was initiated to isolate and characterize dominant proteases in the surf clam viscera for possible industrial applications to upgrade the economic value of this by-product, since proteases are of increasing importance in industrial applications (Maugh II, 1984).

MATERIALS & METHODS

Crude enzyme extract

About 200–250g clam bellies obtained from a clam processing company in Long Island were mixed with twice that weight of 4°C water and homogenized in a commercial Waring Blendor at high speed for 2 min. The homogenate was then centrifuged at 4°C and 13,000 × *g* for 30 min. The supernatant was used as a crude enzyme extract for further study.

Purification of clam acid proteases

Clam acid proteases were purified according to the following scheme developed through the course of this study: crude enzyme extract was acidified to pH 3.2 with 2N HCl, and after the removal of precipitates by centrifugation (13,000 × *g*, 4°C, 10 min) the acidified supernatant was stabilized by the addition of 10 mM cysteine-HCl and 2.5 mM Na_2EDTA. The pH of the supernatant was immediately adjusted back to 4.2 with 2N NaOH. Five-hundred milliliters of this acidified-stabilized extract were concentrated 25 times by ultrafiltration using XM-50 membrane (Amicon Co., Lexington, MA) in an Amicon TCF-10 batch ultrafiltration unit operated at 4°C, 35 psig and 200 mL/min recirculation rate. Chilled ethanol was then slowly mixed with the enzyme concentrate until the content of ethanol in the mixture was 40% by volume. The mixture was centrifuged (13,000 × *g*, 4°C, 10 min) to remove the precipitate. The ethanol content of the supernatant was brought up to 70% by volume and the precipitate recovered by centrifugation (13,000 × *g*, 4°C, 10 min). The second precipitate (40–70% ethanol fraction) was redissolved and chromatographed on a Bio-gel P-150 column (2.2 × 75 cm) at room temperature using 0.05M citrate-phosphate buffer containing 10 mM cysteine-HCl and 2.5 mM Na_2EDTA at pH 4.2. The most active fractions eluted from the column were collected and frozen at −20°C for later analysis.

Proteolytic activity determination

The activity of proteolytic enzymes was measured by Anson's method (1938) or Kunitz's method (1947) with modifications.

Substrates. Purified casein 2.5g or 5.0g bovine hemoglobin, both from Sigma Co. (St. Louis, MO), was slowly dissolved in 100 mL of hot 0.015N HCl and diluted to 250 mL to make 1% casein or 2% hemoglobin solution, unless otherwise mentioned. In addition to casein and hemoglobin, bovine serum albumin, fibrin, fibrinogen, elastin and bovine tendon collagen were used to test the general proteolytic activity of the enzymes.

Buffers. HCl-KCl, citrate-phosphate and Tris-HCl buffers, all hav-

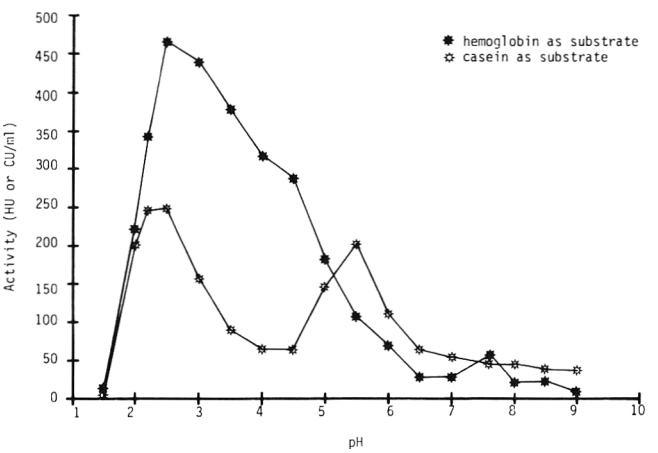

Fig. 1—Effect of pH on proteolytic enzymes in the crude extract from clam processing waste (HU = hemoglobin unit; CU = casein unit).

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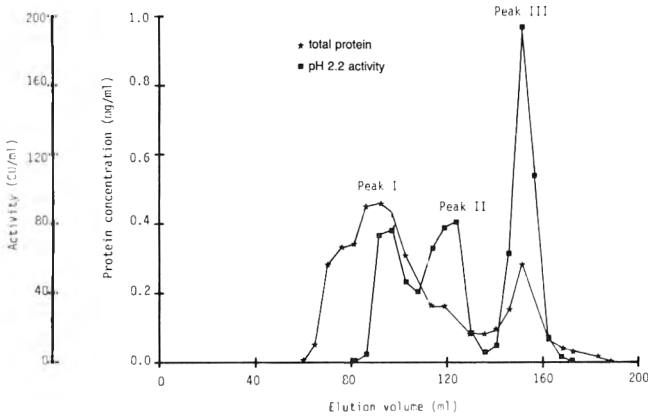

Fig. 2—Biogel P-150 filtration chromatogram of 40–70% ethanol fraction of enzyme extract (CU = casein unit).

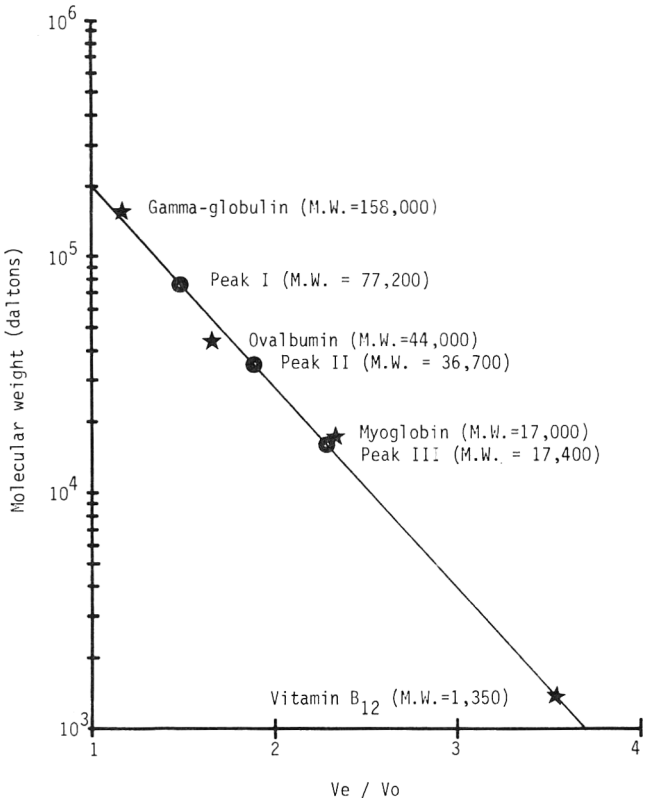

Fig. 3—Molecular weight estimation of peak I, II and III by separation on calibrated Biogel P-150 gel chromatography column.

Table 1—Purity and yield of peak I, II and III proteases in each purification step (CU = casein unit)

Fraction	Protein (mg)	Total activity (CU)	Specific activity (CU/mg)	Yield (%)
Water extract	3,910	68,302	17.5	100.0
Acidified-stabilized extract	845	72,334	85.6	105.9
25 × UF concentrate	576	67,677	117.5	99.1
40–70% ETOH fraction	177	51,010	288.2	74.7
Biogel P-150 column				
Peak I	45	8,104	180.1	11.9
Peak II	18	8,086	449.2	11.8
Peak III	23	14,004	596.0	20.5

ing a concentration of 0.2 M, were used in the pH range 1.3 to 1.9, 1.9 to 7.0, and 7.0 to 10.0, respectively.

Assay procedure. To 0.50–0.25 mL enzyme solution was added

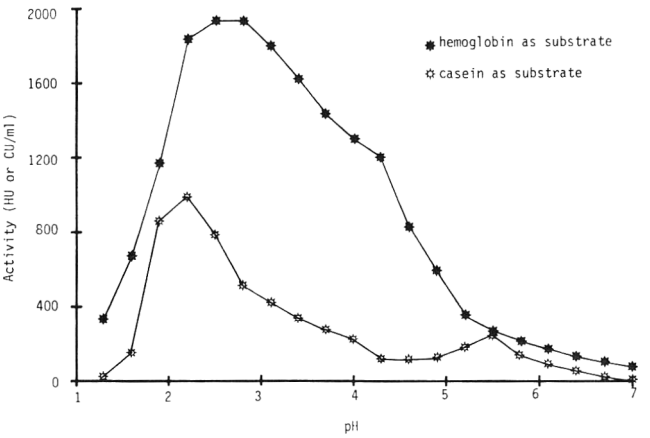

Fig. 4—pH activity curves of purified peak II protease (HU = hemoglobin unit; CU = casein unit).

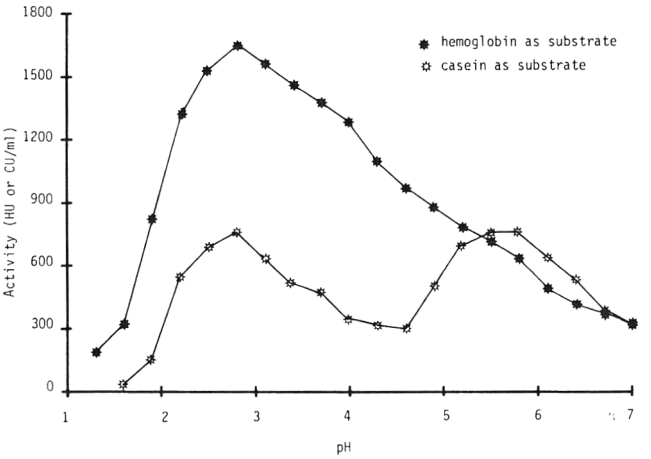

Fig. 5—pH activity curves of purified peak III protease (HU = hemoglobin unit; CU = casein unit).

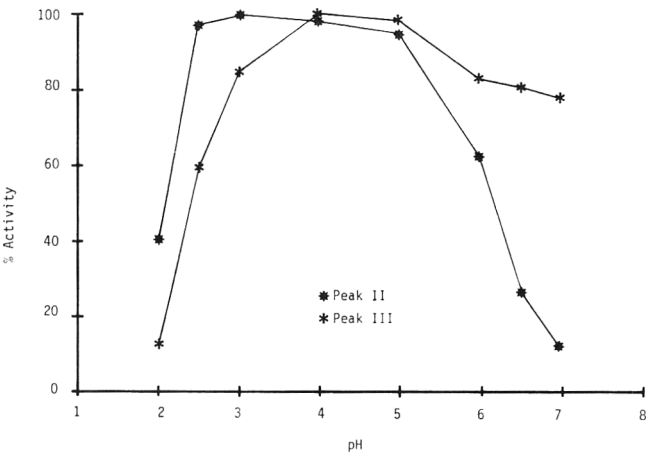

Fig. 6—pH stability of purified peak II and III proteases in hydrolyzing 1% casein solution.

1.95–1.75 mL 0.2M buffer solution, followed by 1 mL substrate. The mixture was incubated at 37°C for 15 min before the addition of 5 mL 0.2M trichloroacetic acid (TCA) to precipitate undigested protein. The 15 min incubation time was selected by running a time course of the assay. Within this time interval the reaction is zero order. After another 10 min, the mixture was filtered through Whatman No. 42 paper. The amount of TCA soluble peptides produced by enzymic digestion was measured spectrophotometrically at 280 nm. A blank was run simultaneously with each determination by reversing the addition order of substrated and TCA.

Table 2—Effect of metal ions on the proteolytic activity of peak II and III proteases

Metal ions (0.5 mM)	Peak II		Peak III	
	Activity (HU/mL)[a]	% Retention	Activity (HU/mL)[a]	% Retention
Control	214.7	100.0	258.7	100.0
Ca^{++}	209.4	97.5	256.7	99.2
Mg^{++}	211.4	98.5	254.0	98.2
Sn^{++}	190.6	88.8	216.5	83.7
Pb^{++}	214.0	99.7	250.7	96.9
Zn	194.0	90.4	266.7	103.1
Cd^{++}	206.7	96.3	249.4	96.4
Hg^{++}	187.0	87.1	0.0	0.0
Cu^{++}	205.4	95.7	46.7	18.0
Ag^{++}	219.3	102.2	245.8	95.0
Ni^{++}	206.0	96.0	239.4	92.5
Co^{++}	208.0	96.9	234.7	90.7

[a] HU = hemoglobin unit.

Table 3—Effect of protease inhibitors on the proteolytic activity of peak II and III proteases

Inhibitors	Final conc	% Residual activity	
		Peak II	Peak III
Pepstatin A	1 μg/mL	25.0	99.4
Poly-L-lysine	1 mg/mL	99.3	101.1
PMSFP[a]	1 mM	102.2	53.5
Iodoacetamide	1 mM	98.9	78.3
TPCK[b]	1 mM	96.4	21.2
TLCK[c]	1 mM	96.2	16.8
Soya trypsin inhibitor	1 mg/mL	100.5	87.4
Ovomucoid trypsin inhibitor	1 mg/mL	98.0	97.4
Leupeptin hemisulfate	1 μg/mL	96.5	33.4
Antipain di-HCl	1 μg/mL	102.9	89.7
DNME[d]	1 mM	100.0	64.9

[a] phenylmethanesulphonylfluoride
[b] tosyl-phenylalnine chloromethyl ketone
[c] tosyl-lysine chloromethyl ketone
[d] diazoacetylnorleucine methyl ester

Table 4—Relative activity of peak II and III proteases towards different substrates

Substrate (1%)	Relative acidity (%)	
	Peak II	Peak III
Hemoglobin	100.0	100.0
Casein	30.3	23.8
Bovine serum albumin	26.0	34.7
Fibrinogen	34.2	27.2

Definition of activity units. The activity of proteolytic enzymes was expressed in casein units (CU) or hemoglobin units (HU). One CU or HU was arbitrarily defined as 0.001/min increase in A_{280} under assay conditions using casein or hemoglobin as substrate, respectively.

Protein concentration determination

Concentration of protein in each enzyme preparation was measured on the basis of the dye-binding assay developed by Bradford (1976) and described in the Bio-Rad Instruction Manual 82-0275-1282 (Bio-Rad Laboratories, Richmond, CA).

pH and temperature stability test

pH stability was determined by preincubating 0.05 mL gel-filtration-purified enzyme solution in 1.95 mL 0.2M HCl-KCl, citrate-phosphate or tris-HCl buffers of different pH at 37°C for 60 min before the addition of 1% casein substrate in the proteolytic assay. Temperature stability was determined by preincubating 0.05 mL gel-filtration-purified enzyme solution at 37° and 57°C for different time periods at pH 3.0 (cathepsin D-like protease) or 4.0 (cathepsin B-like protease) before the proteolytic assay. Each milliliter of gel-filtration-purified enzyme solution contained 0.2–0.4 mg proteins (Fig. 2).

Heavy metal test

The effect of heavy metals on proteolytic activity was determined by preincubating a mixture of 5 mM heavy metal stock solution (0.3 mL), 0.2M citrate-phosphate buffer at pH 2.8 (1.6 mL) and gel-filtration-purified enzyme solution (0.1 mL) at room temperature for 10 min before the addition of 2% hemoglobin substrate. All stock solutions of heavy metals were made from chloride salts, except that silver was prepared from the nitrate salt.

Inhibitor test

The effect of inhibitors on proteolytic acitivity was determined by preincubating a mixture of inhibitor stock solution (0.5 mL), citrate-phosphate buffer at pH 2.8 (1.7 mL) and gel-filtration-purified enzyme solution (0.25 mL) for 10 min at room temperature before the addition of 2% hemoglobin substrate. All inhibitors were from Sigma Co. (St. Louis, MO). The stock solutions were prepared by dissolving the inhibitors either in water or dimethyl sulfoxide (DMSO) as follows: Water-Leupeptin hemisulfate (60 μg/mL), Antipain (60 μg/mL), Poly-L-lysine (M.W. 4,000–15,000) (60 mg/mL), soybean trypsin inhibitor (60 mg/mL), ovomucoid trypsin inhibitor (60 mg/mL), Tosyl-lysine chloromethyl ketone (TLCK, 60 mM) and iodoacetamide (60 mM). DMSO-Pepstatin (60 μg/mL), Tosyl-phenylalanine chloromethyl ketone (TPCK, 60 mM), phenylmethanesulphonylfluoride (PMSF, 60 mM), and diazoacetylnorleucine methyl ester (DNME, 60 mM).

Activity against BAPNA

The activity of gel-filtration-purified enzyme fractions against synthetic substrate, α-Benzoyl-DL-arginine p-nitroanilide (BAPNA) was measured by the method of Arnon (1970) with modifications. Forty milligrams of BAPNA were dissolved in 2 mL DMSO and diluted to 40 mL with water. A mixture of purified enzyme solution (0.1 mL), 0.2M citrate-phosphate buffer (1.9 mL) and BAPNA solution (2 mL) was incubated at 37°C for 60 min. The reaction was stopped by the addition of 1 mL 33% acetic acid and the amount of p-nitroanilide released was measured at A_{410} nm against blank. One BAPNA unit (BU) is defined as a 0.001 increase of A_{410}/min under assay conditions.

RESULTS & DISCUSSION

pH-activity profile of clam viscera extract

The relationship between pH and proteolytic activity of crude extract prepared from surf clam viscera is shown in Fig. 1. Two activity peaks, one at pH 2.2 and another at pH 5.5, were observed when casein was used as substrate. However, only one activity peak at pH 2.8 was observed when hemoglobin was used as substrate. Since the position of the pH optimum may be influenced by the ionic strength of the medium (Douzou and Maurel, 1977) and by the nature and conformation of the substrate (Schlamowitz and Peterson, 1959), it was not obvious whether the pH 5.5 peak was a distinct protease from the pH 2.2 (pH 2.8 for hemoglobin) peak or there was just one protease showing multiple pH optima. The situation was further complicated by the low solubility of casein near its isoelectric point at pH 4.6. Further investigation was oriented to recover the pH 2.2 (2.8) activity peak, because it was the most dominant protease in the crude extract of clam viscera. The protease activity was measured at pH 2.2 with casein or pH 2.8 with hemoglobin during further purification.

Purification of clam proteases

When crude extract was purified according to the scheme described in the materials and methods, three peaks, all active at pH 2.2, emerged from Biogel P-150 filtration column. For the sake of convenient reference, these three peaks were designated as PI, PII and PIII, respectively (Fig. 2). The most active fractions (as indicated by the horizontal bar) of each peak were collected for further investigation. From their elution volumes, the molecular weights of PI, PII and PIII were, respectively, estimated to be 77,200, 36,700, and 17,400 daltons using the same gel filtration column calibrated with known molecular weight markers (Fig. 3).

It was found that in the initial gel filtration trials only PI and PII peaks appeared in the eluent, when cysteine and EDTA

Table 5—Summarized properties of peak II and III proteases as compared with cathepsin D[a] and B[b]

Properties	Peak II	Cathepsin D	Peak III	Cathepsin B
Molecular weight (daltons)	36,700 (77,200)[c]	42,000 (100,000)[d]	17,400	24,000
pH optima				
casein	2.2	?	2.8, 5.5	6.0
hemoglobin	2.8	2.8–3.5	2.8	4.0–4.5
pH stability	2.5–5.0	unstable at pH > 7	3.0–7.0	unstable at pH > 7
Temperature optima	44–48°C	45°C	42–46°C	40°C
Temperature stability				
37°C, 90 min	86.7% (pH 3)	?	100% (pH 4)	?
57°C, 90 min	3.8% (pH 3)	?	71.4% (pH 4)	?
Inhibitors				
Heavy metal				
Cu^{++}	–	–	+	+
Hg^{++}	–	–	+	+
Pepstatin	+	+	–	–
Leupeptin	–	–	+	+
DNME w/Cu^{++}	–	+	+	+
TLCK	–	–	+	+
TPCK	–	–	+	+
Activators	none	none	EDTA Cys-HCl	EDTA Cys-HCl
Attack BAPNA[e]	–	–	+	+

[a] Barret (1977c); Takahashi and Tang (1981)
[b] Barrett (1977b); Barret and Kirschke (1981)
[c] Molecular weight of peak I.
[d] High molecular weight form of cathepsin D
[e] α-Benzoyl-DL-arginine p-nitroanilide

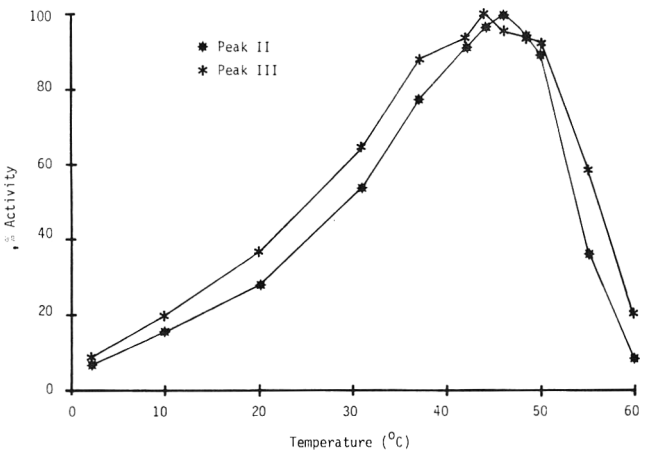

Fig. 7—Temperature activity of purified peak II and III proteases in hydrolyzing 1% casein solution.

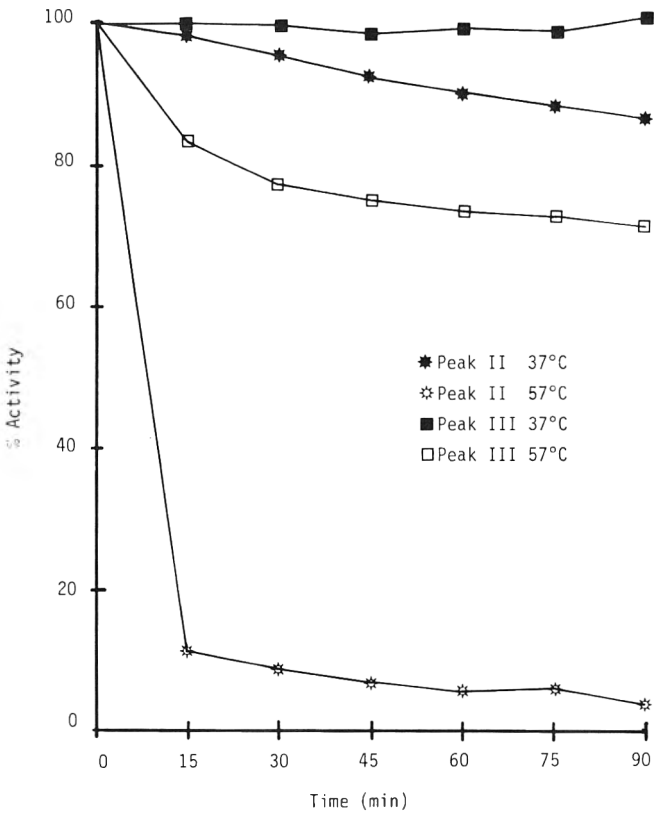

Fig. 8—Thermostability of purified peak II and III proteases in hydrolyzing 1% casein solution.

were omitted in the elution buffer. PIII was completely inactivated throughout gel filtration process. The addition of EDTA and cysteine to the PIII fraction activated the enzyme, but had no effect on PI and PII fractions. The addition of cysteine and EDTA into elution buffer prevented the loss of PIII activity during filtration. These observations strongly suggest that PIII is a thiol protease requiring EDTA and thiol compounds for maximum activity and stability. The purity and yield of each fraction throughout the whole purification process is shown in Table 1.

Characterization of clam acid proteases

The distinct separation of these three peaks by gel filtration warranted the characterization of these enzymes. PI and PII were found to be similar in many aspects, including temperature and pH profiles, heavy metal and inhibitor effects, and substrate specificity. Molecular weight is the only major difference found between PI and PII. It was suspected that PII is a subunit of PI or PI is a proenzyme of PII. In the following discussion only the data for PII and PIII fractions are discussed.

pH optima and stability. Optimum activity of PII towards casein and hemoglobin occurred at pH 2.2 and 2.5–2.8, respectively (Fig. 4). There was a very small shoulder towards casein at pH 5.5 which was likely an artifact resulting from the insolubility of casein near its isoelectric point. PII had very limited activity at pH above 6. The optimum activity of PIII towards hemoglobin occurred at pH 2.8. However, two activity peaks, one at pH 2.8 and another at pH 5.5–5.8, occurred when casein was used as substrate (Fig. 5). Again, low activity towards casein was observed near its isoelectric point. PIII was more active than PII at higher pH. The implication of this

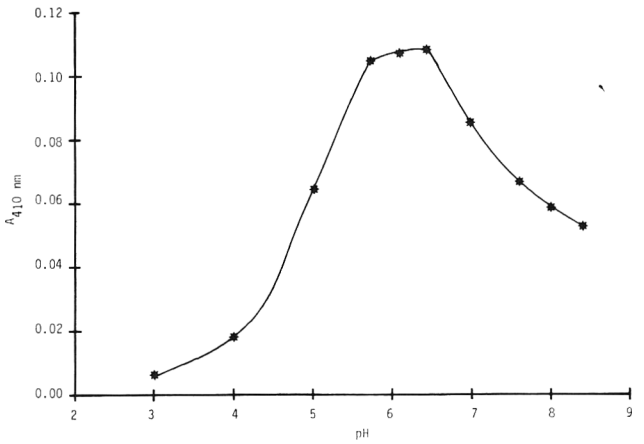

Fig. 9—pH-activity profile of peak II protease towards synthetic substrate α-Benzoyl-DL-arginine P-nitroanilide.

observation is that the activity peak at pH 5.5 found in the crude extract was mainly contributed by PIII. Figure 6 shows that PII was stable (greater than 70% retention of activity) from pH 2.5–5.0 and PIII was stable from pH 3.0–7.0. PIII has much higher pH stability from pH 6–7 than does PII. This difference may be important in the commercial applications of these enzymes.

Temperature optima and stability. Using 1% casein solution as substrate, PII had optimum activity at temperature from 44 to 48°C and PIII exhibited optimum activity at 44–46°C (Fig. 7). Despite their similar temperature optima, PIII appeared relatively more active than PII in both temperature extremes. Fig. 8 reveals that PIII was more stable to heat than PII. The heat stability of PIII might be attributed to the presence of EDTA and/or cysteine in the enzyme solution, because PIII was believed to be a thiol protease (see later sections).

Heavy metal effect. PII was not sensitive to heavy metals (Table 2). In contrast, PIII was very sensitive to cupric ion and extremely sensitive to mercuric ion, a common property of thiol proteases. Neither PII nor PIII is similar to the copper-activated proteases found from *Mytilus galloprovincialis* (Iordachescu et al., 1978). Lead ions did not inhibit PIII under the general assay procedure described in Materials and Methods. However, when the addition order of buffer and inhibitor solution was reversed, only 20.7% of PIII activity was detected for lead ion, but this effect could not be observed in other ions. It appeared that the addition of citrate-phosphate buffer in advance prevented the binding of Pb^{++} to PIII probably due to the binding of Pb^{++} with citrate of phosphate. Although Sn^{++} has been reported to activate thiol proteases, such as papain and *Aspergillus* protease (El-Zalaki et al., 1974), it inhibits PIII slightly. A study of concentration effect of cupric ions showed that complete inactivation of PIII could be achieved at a cupric ion concentration of 1 mM. The activity of PII was not affected up to this concentration of cupric ions. It might be of interest to further investigate the effect of heavy metals on the pH stability and optimum and on the temperature stability and optimum of each enzyme.

Inhibitor effect. As shown in Table 3, PII was strongly inhibited by pepstatin, a pentapeptide with two unusual amino acids, produced by *Streptomyces*. It is probably a powerful inhibitor of all carboxyl proteases, with little or no effect on other classes of proteases (Umezawa and Aoyagi, 1971). This observation suggests that PII is a carboxyl protease. Further evidence is the fact that PII is insensitive to all other inhibitors which commonly inhibit serine and thiol proteases. Carboxyl proteases, such as cathepsin D and pepsin, are also inhibited by DNME in the presence of the cupric ion (Barrett, 1977c). However, PII was not sensitive to DNME in the presence of cupric ions even up to 4 hours of preincubation. On the other hand, PIII was very sensitive to DNME even without the presence of cupric ion. PIII was also inhibited by PMSF, iodoacetamide, TPCK, TLCK, and leupeptin. These observations suggest that PIII is a thiol protease. Despite the fact that most of these inhibitors also react with serine proteases, PIII is not likely being a serine protease, since it was not inhibited by trypsin inhibitors, but strongly inhibited by DNME and needed a thiol compound, such as cysteine, for maximum activity.

Substrate specificity. Proteases attack substrates at varying rates, depending on the nature of substrate and the enzyme. For example, alkaline proteases isolated from sardine stomach hydrolyzed casein more rapidly than hemoglobin (Noda et al., 1982). On the other hand, acid proteases isolated from the same source hydrolyzed hemoglobin more rapidly than casein (Noda et al., 1982). According to Barrett (1967), cathepsin D can be rather easily differentiated from pepsin by the fact that serum albumin is hydrolyzed at 10% or less of the rate for hemoglobin whereas pepsin is equally active against both proteins. To help elucidate the identity of the enzymes isolated from clam viscera, the activity of the three components obtained after gel filtration towards several protein substrates was investigated. Both PII and PIII were more active against hemoglobin than casein, bovine serum albumin or fibrinogen (Table 4). Insoluble substrates, such as collagen, elastin and fibrin, were not degraded by either PII or PIII under the conditions described in the assay of proteolytic activity. PIII could attack α-Benzoyl-DL-arginine p-nitroanilde (BAPNA), a synthetic substrate for papain, bromelain, trypsin and cathepsin B. Purified PIII had a general proteolytic activity equivalent to 650 CU/mg and a specific activity of 65 BU/mg against BAPNA. Optimum pH for this substrate shifted to 6.0–6.5 (Fig. 9). This observation is similar to that of Burleigh et al. (1974) who found that cathepsin B had a lower pH optimum towards protein substrate, but higher pH optimum towards synthetic substrates (Barrett and Kirschke, 1981). PII had no activity towards BAPNA.

CONCLUSIONS

PII is a carboxyl protease. Among the most important carboxyl proteases discovered in mammalian viscera tissues are pepsin, gastricin, chymosin, renin and cathepsin D. A comparison of PII with these enzymes suggests that PII is cathepsin D-like enzyme rather than pepsin-like or gastricin-like enzyme, primarily because it is insensitive to poly-L-lysine (Reid and Rauchert, 1976) and has much greater preference of hemoglobin over serum albumin as substrate (Barrett, 1967). Similarly, a comparison of PIII with thiol proteases, such as cathepsin B, H and L, indicates that PIII is a cathepsin B-like enzyme. PIII differs from cathepsin L, since the latter can not attack BAPNA (Barrett, 1977a, b). PII differs from cathepsin H, since the latter is rather insensitive to leupeptin (Barrett and Kirschke, 1981). The properties of PII, PIII and cathepsin D and B are summarized in Table 5. PII differs from cathepsin D in the sensitivity to DNME in the presence of cupric ion. PIII has a lower pH optimum towards casein and hemoglobin than cathepsin B. PII and PIII are smaller than cathepsin D and B, respectively, but otherwise they are very similar.

The absolute identity of PII and PIII is a difficult problem even if they had been purified to homogeneity. Due to the extreme similarity among cathepsin D, pepsin, gastricin and among cathepsin B, H, and L, we can not totally eliminate the possibilities that the PII fraction contained pepsin or gastricin and PIII fraction contained cathepsin H or L. Further purification and characterization works are required to solve these doubts.

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Chemical and Physical Characteristics of Lightly Salted Minced Cod (*Gadus morhua*)

E. G. BLIGH and R. DUCLOS-RENDELL

ABSTRACT

The production of a lightly salted product from minced cod was investigated. The addition of 13% salt to cod mince showed several advantages over the previously employed 25% salt treatment. The lightly salted product had a gelatinous-fibrous texture which had shape stability after drying and cooking. When dried to 30% moisture, better water binding capacity was seen during rehydration and after cooking, with improved texture as measured on the Instron instrument.

INTRODUCTION

RECENT MEDIA ATTENTION on world hunger, particularly on the African continent, has demonstrated rather dramatically the ongoing need for new and perhaps unconventional food sources. Protein from marine products is nutritionally complete and, in light of increasing economic and environmental pressures to utilize fishery by-catch materials and underutilized species, fish may represent a potential solution for the malnourished populations of the world. One product which may be particularly promising is minced fish flesh. Its production offers several advantages over the more conventional means of using seafoods: (1) provides a higher yield; (2) permits use of filleting waste, shrimp by-catch and nontraditional species; (3) easy incorporation with other foodstuffs to increase nutritional aspects and palatability; (4) cost is fairly minimal; and (5) it is a simple process requiring no special skills and a very short processing time.

An effective method of using minced fish flesh may be salting, since this ensures preservation and actually requires little further processing. Additionally, a salted mince product may substitute for more traditional salt fish, of which production has declined dramatically over the past few decades, in spite of a fairly healthy demand from tropical countries (Bligh and Regier, 1976).

Salting of minced fish was first introduced by Del Valle and Nickerson (1968). Their process involved grinding of shark flesh with salt, followed by further mixing, pressing of the salt minced flesh at 13,790 kN/m^2 (2000 lb/in^2) to remove water and form cakes, and drying the cake, either artificially or naturally. The product was called "quick salted", due to the rapid rate of salt penetration into exposed muscle. Low moisture content and sufficient salt eliminated bacterial action and insured good storage stability at ambient temperatures.

Del Valle and Gonzalez-Inigo (1968) used this process with other species of fish and noted that insufficient salt content resulted in a gelatinous mass which could not be pressed. Excessive salting produced a very brittle cake after pressing. A correlation was made between the minimal amount of salt necessary and the water holding capacity of the fresh fish. Finally, they showed that only negligible amounts of protein were lost in press juice and leaching water.

Wojtowicz et al. (1977) studied several undefined parameters of the earlier salted mince methods and determined that one part salt with three parts minced flesh was most desirable for saturation and maximum dehydration. Salt concentrations in excess of 25% resulted in an excessively salty product. Also, the addition of salt must be rapid to by-pass the intermediate gel stage with its resultant irreversible rubber-like texture. Temperature during mixing was found to influence rate of salting; a temperature of 35°C, accompanied by efficient stirring, was recommended. Removal of brine from the salt minced flesh involved loss of nutritional components such as water soluble proteins, vitamins, and minerals. However, it also eliminated some highly reactive and undesirable components such as pigments, amino acids, peptides, amines, carbohydrates and carbonyls.

Wojtowicz et al. (1977) also noted that salt minced fish could be stored without further drying when: (1) moisture was not higher than 40–45%; (2) storage temperature was 2–8°C; (3) protection against oxygen and light was provided. Further drying was necessary, however, since the product would be stored normally without refrigeration or even at tropical temperatures. A moisture content of 18–22% (water activity 0.68 and 0.72) reduced chances of halophilic bacterial growth at ambient temperatures.

The main disadvantages of the Wojtowicz product were its lack of protein functionality and its unattractive fibrous texture (Bligh and Duclos 1981). The purpose of the present study

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Table 1—Composition of dried (30% moisture) salt minced cod following various salt treatments and its characteristics before drying

Salt Treatment %	Composition Protein%	Salt%	Characteristics (before drying)
5	55	15	Firm protein gel, sticky, translucent, yellowish color.
9	50	20	Gelatinous, sticky, translucent.
13	48	22	Semi-gelatinous, semifibrous, cohesive, slightly white.
17	44	26	Noncohesive, semi-fibrous, white.
20	44	26	Semi-dry fibrous protein, opaque white, friable
25[a]	40	30	Semi-dry fibrous protein opaque white, friable

[a] Wojtowicz et al. (1977)

Table 2—Water uptake during rehydration of dried salt minced cod

Weight (g)	Salt Treatment (%) 5	9	13	17	20
Wt before soaking	28.55	32.53	29.83	28.59	29.14
Wt after soaking[a]	46.99	45.22	54.16	43.94	42.76
% water uptake	39.2	28.1	44.9	34.9	31.9

[a] Soaked 4 hr at approx. 21°C in 10 volumes of water.

Table 3—Composition of salt minced cod after rehydration and cooking

Composition	Salt treatment (%) 5	9	13	17	20
Moisture	66	68	72	67	68
Protein	31	30	26	30	31
Salt	3	2	2	3	1
Total	100	100	100	100	100

was to investigate production of a lighter salted minced cod product that would eliminate these undesirable characteristics of the earlier product. Duerr and Dyer (1952) reported denaturation of cod muscle protein at salt concentration of 8–10% in the muscle. Minced cod with a salt concentration in this range, rather than 25% previously used, should yield a product with greater protein functionality and more desirable physical characteristics.

MATERIALS & METHODS

Raw material

Strictly fresh whole and gutted cod (*Gadus morhua*) were purchased from local suppliers. The fish were split manually, the backbone and kidney were removed, and they were rinsed in clean tap water before being minced. Fine grade fishery salt was used for salting.

Pilot plant production

Split cod were mechanically deboned using a Bibun SDX 16 meat-bone separator (Ryan Engineering Inc., Seattle, WA) fitted with a 5 mm hole drum. The mince was held at 5°C until salting later the same day. Batches of minced cod, weighing approximately 2.1 kg each, were mixed for 5 min in a Hobart mixer with 9,13,17,20, or 25% salt (w/w). The mixture was held at 35°C for 30 min with frequent stirring. Released brine was removed using a vacuum filter press (Passey and Hotton, 1978). The pressed slabs (approximately $61 \times 22 \times 1$ cm) were dried to 30–35% moisture in a mechanical drier with an inlet temperature of 29°C and relative humidity of 60%. Drying time ranged from 28–54 hr, depending upon the salt treatment. The dried product was held for 24 hr at 5°C to permit moisture equilibration within the slab. Finally, the dried salt minced cod was cut into cakes approximately 8 cm^2 and sealed (without vacuum) in laminated polyethylene-aluminum foil pouches.

Laboratory analyses

Composition of the various salted minced products was determined in triplicate. Protein was analyzed by the micro-Kjeldahl method for nitrogen (Concon and Soltess, 1973). Sodium chloride levels were determined by conductivity (Duclos-Rendell, 1983). Moisture was determined by measuring weight loss of a 10-g sample dried for 24 hr at 105°C.

Texture analysis was performed on mince which was desalted and rehydrated by soaking a 30-g sample in 10 volumes of water for 4 hr. Excess water was then drained in a Buchner funnel and percentage water uptake calculated from weight difference before and after rehydration. Samples were cooked in 75 mL boiling water for 4 min, and texture analyzed using the Instron Universal Testing Instrument (Gill et al., 1979).

A Gardner Automatic Color Difference meter (Model AC-3, Gardner Laboratory, Bethesda, MD) was employed to analyze color in cooked and cooled mince samples which were pressed to the bottom of a glass petri dish in order to be as flat as possible. These were rotated and measured three times. Average lightness (L) values (where 0 is black and 100 is white) and standard deviations were calculated.

RESULTS & DISCUSSION

THE WATER HOLDING CAPACITY of the minced cod following the various salt treatments is presented in Fig. 1. These results confirmed that the water holding capacity of cod flesh decreased with an increase in salt concentration. A rapid increase in water loss was observed when more than 9% salt was reached in the muscle, demonstrating that salt treatments of less than 25% can be used to denature the protein and to release appreciable quantities of water from the flesh. The 13% salt treatment was very effective, removing about one-half of the water that was released by the 25% salt treatment, while retaining some functional properties of the protein.

Composition of the salted minced products is reported in Table 1. At a common moisture level of 30%, protein values decreased inversely with increased additions of salt as expected. Compared to the product of Wojtowicz et al. (1977), 13% salt treatment yielded an end product with appreciably more protein and less salt. Furthermore, it retained a formed

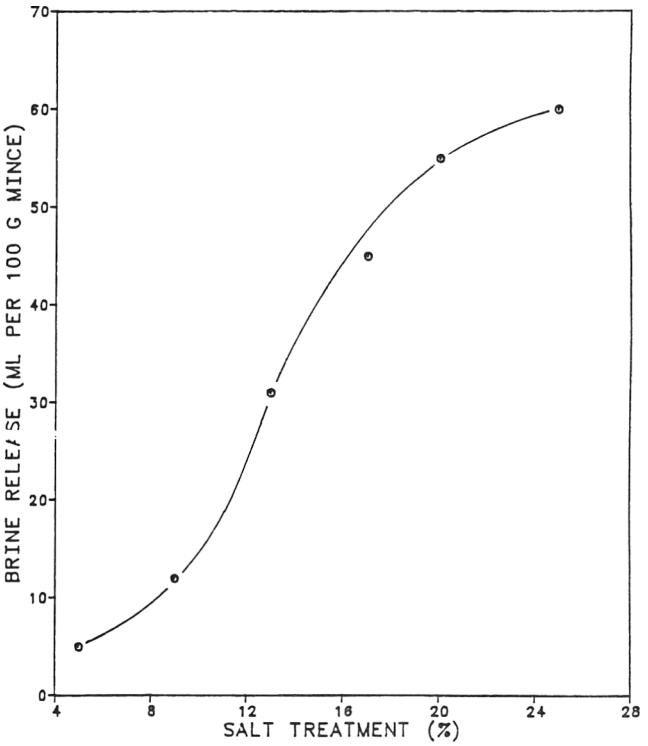

Fig. 1—Brine released (by filter press) versus salt treatment of the minced cod muscle.

Table 4—Textural characteristics of the cooked salt minced cod as measured on the Instron instrument

Salt Treatment	Peak height Force (kg)[a]	Peak slope (kg/cm)	No. of samples
25	350.3 ± 8.4 (S.D.)	28.74 ± 2.00	6
20	282.7 ± 4.5	17.62 ± 6.00	3
17	205.3 ± 18.1	16.34 ± 1.94	10
13	147.3 ± 17.7	8.26 ± 1.02	10
9	137.2 ± 8.0	7.63 ± 1.25	10

[a] The average peak height for fresh cod samples after cooking was 66.2, where the average peak slope was 19.8.

shape (i.e., a cake) and had an acceptable semi-fibrous texture which could withstand handling after drying. Conversely, Wojtowicz's product was described as friable and somewhat mealy.

Protein functionality of the salt treated products was studied in relation to their water uptake during rehydration and cooking (Table 2). Water uptake was not proportional to the amount of salt added. The results indicated that the 13% salt product absorbed more water (by weight) than the others, showing an improvement in water binding capacity.

Composition of the various products was re-examined after cooking (Table 3). Rehydration and cooking removed most of the salt, making the product acceptable for consumption. Of more significance, however, was the fact that the 13% salt product retained its water holding capacity and shape stability through cooking.

Results for Instron texture measurement are shown in Table 4. Textural deterioration is characterized by higher peak heights and steeper peak slopes. The texture values showed that increasing amounts of salt contributed to toughening. Although the 9% salt product was the most tender, its gelatinous characteristics were undesirable in that they inhibited moisture removal and produced a rubbery product. This appeared to be overcome by the addition of about 13% salt which still yielded a product with better texture than higher salt treatments.

The appearance of the cooked products was also noted. The 9% salt treatment was rubbery and wet; the 20 and 25% salt treatments were fibrous (mealy) and dry; the 13 and 17% salt

Table 5—Mean color values (L)[e] for cooked salt minced cod products[f]

	Salt treatment (%)				
	5	9	13	17	20
Initial Product	58.95 ± 0.10[d]	60.82 ± 0.87[bcd]	65.57 ± 0.8[a]	62.45 ± 0.91[b]	61.88 ± 1.11[bc]
Stored Product[4]	62.95 ± 1.19[c]	65.29 ± 1.84[b]	68.55 ± 1.28[a]	64.09 ± 0.66[bc]	67.97 ± 075[a]

[a d] Values are means of three determinations, plus or minus standard deviation. In each row, values with the same superscript are not significantly different (P = 0.05).
[e] L = lightness, where 0 is black and 100 is white.
[f] Product stored 2 months approx. 21°C.

treatments had more textured structures resembling traditional salt cod.

The color of the products was measured in relation to salt content and storage for 2 months at approx. 21°C (Table 5). The results indicated that the lighter salt treatment at 13% produced a product as white as the higher salt treatments and that this characteristic was maintained during limited storage at ambient temperature.

It was found that the typical odor and taste of salted cod developed more rapidly when the mince was dried immediately after salting and was subsequently stored at about 21°C for at least 9 days. Prolonged storage prior to drying did not induce flavor and odor development which appeared to be promoted by the drying process.

This study on light salting of minced cod revealed that improvements can be made on the previous heavy salted products. The water binding properties of minced cod tissue were significantly altered by the addition of 13% salt to enable the production of a product with improved protein functionality. Although 25% salting normally released twice as much tissue-bound water as 13% salt treatment, the latter product had better rehydration capacity and shape retention even after cooking. Moreover, the texture of the light salted material was more like that of traditional salt cod.

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Comparison of Whole with Headed and Gutted Orange Roughy Stored in Ice: Sensory, Microbiology and Chemical Assessment

D. N. SCOTT, G. C. FLETCHER, M. G. HOGG, and J. M. RYDER

ABSTRACT

Sensory, microbiological and chemical analyses were used to compare whole orange roughy stored in ice with headed and gutted orange roughy stored in ice. Sensory results indicated that the whole fish had a shelf-life of 11 to 13 days. Heading and gutting gave only a slight increase in storage life. Microbiological results suggested that this increase was not due to any reduction in bacterial activity. However, chemical analyses indicated that there was reduced autolysis in the headed and gutted fish. Sulfide producing bacteria were not a significant proportion of the spoilage flora. The K value and results of analyses of inosine 5'-monophosphate and inosine were consistent with the sensory results and therefore these chemical tests are considered useful for monitoring changes in the freshness of orange roughy during ice storage.

INTRODUCTION

IN 1978 New Zealand declared its 200 mile Exclusive Economic Zone and so became responsible for the management of the fish resource within the area. The zone includes the Chatham Rise, a deep water continental shelf which contains large stocks of fish. One such fish is the orange roughy (*Hoplostethus atlanticus*), now one of New Zealand's major fish export earners. Present indications are that the fishery has good long term prospects (Robertson and Grimes, 1983).

In the initial stages of the development of this resource, fish were landed by foreign fishing vessels in the frozen headed and gutted form. However, in June 1981 the first catch was made by a New Zealand boat which landed the fish whole in ice (Anon., 1981). There has been a large increase in the quantity of orange roughy landed in ice over the ensuing period and on occasions the physical appearance of the fish has been indicative of considerable quality loss.

To date, research into the handling and processing of orange roughy has been limited. The oil, protein, moisture and ash contents of the flesh of orange roughy have been previously reported (Vleig, 1983). Also Buisson et al. (1982) have examined the properties of the oil. Grigor et al. (1983) have studied the composition and location of the oil in the fish.

Research workers have reported an extension in shelf-life as a result of gutting fish such as gurnard (Vyncke, 1980), Patagonian hake (Lupin et al., 1980), spiny dogfish (Bilinski et al., 1983) and several underused Gulf fishes (Waters, 1978). Botta et al. (1982) reported that gutting reduced dimethylamine production in roundnose grenadier during frozen storage. Burt et al. (1974) demonstrated that delays in the gutting of Cape hake led to discoloration and gaping in subsequently thawed fillets. Boyd and Wilson (1977) found that gutting reduced the rate of loss of texture quality in snapper caught during the period when the fish are actively feeding. Huss and Asenjo (1976), however, noted that while all fish they examined suffered some loss of quality if gutting were omitted, great differences were found between fish species. Other workers have reported that gutting did not significantly extend the shelf-life (Maia et al., 1981; Adebona, 1981; Avdalov and Ripoll, 1981). The combined effect of heading and gutting on shelf-life of fish species has not been widely reported.

The purpose of this study was to compare the storage in ice of whole orange roughy with headed and gutted orange roughy by sensory profiling and chemical and microbiological analyses. Sensory profiling was used as it has been shown to provide useful descriptive information about quality changes (Quarmby et al., 1982; Bremner and Statham, 1983).

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MATERIALS & METHODS

Sample preparation

Orange roughy used for this study were caught in November 1982 by bottom trawling at a depth of 950–1050m in Area D (approx. position: Long 174 30 E, Lat 43 S) of the New Zealand Exclusive Economic Zone. Fish of good visual quality were removed from the net and treated in one of three ways: (1) seventy fish were washed with seawater and packed whole (W) into fish bins with an equal volume of ice; (2) a further 70 fish were headed and gutted (HG), washed with seawater and packed into fish bins with an equal volume of ice; (3) sixty fish were stored whole in an ice-seawater slurry for 12 hr. These (post rigor) fish were then filleted, deep-skinned, washed with seawater and frozen to −40°C in a shatter pack carton to provide reference (R) samples for sensory evaluation.

On landing, the iced fish were repacked with an equal volume of ice into plastic-lined polystyrene containers and transported to the laboratory. Upon arrival they had been in ice for a total of 3 days. The fish were then placed in a large insulated container where the ice:fish ratio was maintained at 1:1 for the duration of the trial. The frozen reference fillets were stored at −30°C in the laboratory.

Sampling

Ten fish from W and HG treatments were randomly sampled on days 0, 4, 6, 9, 11, 13, and 16. One fillet from each of five fish was used for microbiological analysis. The other fillet was used for sensory evaluation of cooked fish. The remaining five fish were used for sensory evaluation of raw fish prior to filleting; then one fillet of each fish was used for chemical analysis while the other fillet was used for sensory evaluation of cooked fish.

Sensory evaluation

Raw fish. The color, surface slime, appearance of gills, odor of gills and the texture of the flesh were examined from day 4 until day 13 by two experienced members of the research staff.

Descriptive analysis. Concurrently, the attributes of cooked orange roughy were evaluated by 12–20 panelists (none members of the research staff) who were experienced in the descriptive evaluation of fish (Hogg and Scott, 1984). At each sampling time each panel member evaluated three samples: first R, and then in random order W, and HG samples.

To prepare the sample, approximately 100g of fish were steamed in individual, lidded glass casserole dishes for 12 min and served at about 60°C. Frozen reference samples were thawed and then prepared in the same way. Evaluations were conducted in individual booths, one sample being presented at a time. Panelists used either lemon barley water or reverse osmosis water throughout the trial to clear the palate before and between samples. Prior to each tasting, participants were informed that they were to taste and evaluate three samples of cooked orange roughy. No additional information was provided, and there was no discussion about the samples either during or after the evaluation. The sensory evaluation form had been developed with some assistance from the panelists (Hogg and Scott, 1984).

Panelists described the odor and flavor by selecting an appropriate descriptive term aided by a list of 80 odor terms and 50 flavor terms. An intensity rating was assigned to each selected term where a score of three corresponded to strong, two to moderate and one to weak. Texture was described by considering the intensities of selected attributes at three stages of eating: first three bites, midway through chewing, and immediately prior to swallowing. Mean scores were analyzed by ANOVA (Hogg and Scott, 1984).

Subjective analysis

The acceptability of odor, flavor, texture and the overall acceptability were assessed by drawing marks across 15cm noninterval lines. These lines were anchored 'extremely unacceptable' 0.5 cm from the left-hand end and 'extremely acceptable' 0.5 cm from the right-hand end. The center point was marked with a short perpendicular line. Subjective scores were determined by the position on the line from 0 on the left to 15 on the right. Mean scores were compared statistically using ANOVA.

Microbiological analyses

Individual samples from the flesh and surface of the anterio-dorsal region were taken from five W fish. As the handling procedures for the two treatments were unlikely to differently affect the skin of the anterio-dorsal region, only the flesh was sampled for the HG fish. For the surface sample, 10 cm^2 of skin was swabbed with two sterile swabs, the first of which had been wetted with peptone water (0.1% Difco). The swabs were then shaken 100 times in a 125 mL Wheaten Media bottle containing 100 mL of peptone water. For the flesh samples, approximately 64 cm^2 of skin was first rinsed with 70% ethanol and flamed. This was then aseptically removed and 10 cm^2 of the underlying flesh was sampled to the bone (approx 10g) using a sterile scalpel and forceps. The flesh sample was diluted tenfold (w/w) in peptone water and homogenized for 1 min with a Stomacher 400.

Pour plates were made for aerobic plate counts (APC) using 1 mL of appropriate dilutions (0.1% peptone water) in the "Medium B" described by Simidu and Hasuo (1968). Pour plates for sulfide producer counts were made in the Peptone-Iron agar of Sumner and Gorczyca (1981). All plates were counted after 4 days aerobic incubation at 25°C. Black colonies on the Peptone-Iron agar were recorded as sulfide producers. The treatments were compared using analysis of variance on the $\log_{10}$ transformation of the counts.

Chemical analyses

The pH was measured directly on each fillet using a surface pH electrode. Five sites were chosen on the skeleton side of each of the five fillets and the results averaged.

The anterio-dorsal region of each fillet was subsequently removed, placed in a plastic bag and frozen at −70°C until further analysis. Five grams of frozen muscle were prepared for analysis of nucleotides by high pressure liquid chromatography according to the method of Ryder et al. (1984). Baseline separation of adenosine 5'-triphosphate (ATP) and its degradation products was accomplished within 20 min (Ryder, 1985). K value, as defined by Saito et al. (1959), was also determined. Analysis of variance was carried out on results of each chemical test.

RESULTS & DISCUSSION

Sensory results

Raw fish. Descriptions of the color, surface slime, appearance of the gills, odor of the gills and texture of the flesh have been summarized in Table 1 for the whole fish. After 9 days in ice the skin was blotched possibly due to some orange color having been leached by the ice. The gills remained dark red, with only a slight milky slime and had a sweet, briney, fish-meal-like odor. The flesh was firm and resilient.

After 11 days the head and body of four fish were blue-grey with tinges of orange. The fifth fish was still red-orange in color. The gills were generally brownish-red or bleached with some sticky, creamy slime and had sweet, mussel, soapy, and oily odors. The flesh was slightly soft and retained a slight indentation when pressed.

The changes observed in the sensory attributes of the raw fish (Table 1) were sufficient to provide a suitable means of monitoring quality of whole orange roughy or estimating the time since catching.

Cooked fish. Mean scores for the acceptability of the cooked flesh are shown in Table 2. Scores for all acceptability ratings were consistently higher for the reference samples than those for the fish stored in ice (W or HG) except in one case (texture of HG on day 9) when it was equal. This difference was significant in most instances for flavor and overall acceptability. In part this might be explained by the fact that the panelists were always given the reference sample first. This "position effect" has been observed in other panels (Eindhoven et al., 1964).

Significant differences between W and HG fish occurred on day 13 when both treatments were approaching the end of the storage period. At this stage, the HG fish was considered by the panel to be more acceptable for flavor and overall acceptability than the whole fish.

The acceptability of cooked orange roughy was more dependent on flavor than on texture or odor. The importance of flavor to acceptability of other fish species has been noted by Connell and Howgate (1969) and Rasekh et al. (1970).

Evaluation of the W and HG fish on day 16 by the panel organizer revealed both were putrid so they were not presented to the taste panel.

Description of cooked fish. The description of the odor of cooked orange roughy was similar for all three treatments throughout the trial. The odor of cooked fish was therefore a character not suitable for monitoring changes in orange roughy during storage in ice or differences due to heading and gutting. The odor of the reference sample was consistently described as briney, salty, seaweedy, sweet, and metallic. Shellfish, boiled potato, and fresh milk were also used frequently.

The mean texture scores for W and HG samples were not significantly different from each other or from those of the reference sample throughout the storage period. It has been noted by Love (1966) that taste panels found the description of texture changes to be more difficult than the assessment of flavor changes in frozen fish. It was concluded that the assessment of cooked texture was not a useful measure of quality nor a means of comparing differences due to heading and gutting.

In contrast to odor and texture, the flavors of cooked fish did show changes during storage. A total of 33 different flavor terms was used by tasters to describe the reference sample. Subsequent to the trial, discussions with the taste panelists established that 'creamy' and 'milky' were identical terms as were 'bland' and 'neutral', and 'meaty' and 'lamby'. These terms have therefore been combined and appear as milky/creamy, neutral/bland and lamby/meaty. The terms used by at least 17.5% of the panelists to describe the flavor of the orange roughy samples resulting from each treatment are shown in Table 3. These terms or combined terms were used by at least two panelists at any sampling time. Mean intensity scores for attributes ranged from 1.0–2.5 but showed no trend and were therefore not used in the analysis of results.

The flavor of the reference sample was consistently described as milky/creamy, sweet, lamby/meaty, salty, nutty and buttery. This did not change markedly over the storage period and provided evidence that the panelists, who were not aware of a reference sample, were consistent in their assessment of cooked flavor. Like the reference sample, the W and HG samples were frequently described as sweet, salty, milky/creamy, and lamby/meaty throughout the storage period.

The samples stored in ice (W and HG) were also described as bitter, sour and neutral/bland on more than two occasions while these terms were infrequently used to describe the reference sample. This may have accounted for the lower flavor acceptability ratings given to the two ice-stored samples. For the W and HG samples the most frequently used terms to describe the flavor were similar up to day 11. On day 13, however, the W fish had developed mushroom, cardboard,

Table 1—Sensory assessment of whole orange roughy stored in ice for 4–16 days[a]

Attribute	Days on ice 0	4	6	9	11	13	16
Color	red/ orange	red/ orange	orange on fins, head and tail	sl.blotched body faded	blue steel-grey with tinges of orange	bleached pale grey/blue tail with head pale orange	washed out grey/blue with pale head
Surface slime	no slime	clear	clear or sl.cloudy	clear or sl.cloudy	clear or sl.cloudy	brown slime on body	thick yellow slime
Appearance of gills	dark red	dark red	dark red	dark red slight milky slime	brown/red or bleached sticky creamy slime	brown/red or bleached with brown slime on gills	brown or bleached with brown slime
Odor of gills	sl. seaweedy	sl. seaweedy	mild and sweet	fish meal sweet salty briney	sweet salty mussel soapy oily	metallic stale seaweedy slightly rotting	strong rotting putrid
Flesh texture	firm and resilient	firm and resilient	firm and resilient	firm and resilient	retains finger indentation no gaping sl. soft	retains finger identation soft	retains finger indentation no gaping soft

[a] Evaluations by two people in the laboratory except day 0 which was evaluated by a technician on board the fishing vessel. sl = slightly.

Table 2—Mean acceptability scores for cooked orange roughy

Days in ice	Reference				Whole (W)				Headed and gutted (HG)			
	0[e]	T	F	All	0	T	F	All	0	T	F	All
4	11.4	11.7	12.0	12.0	10.0	10.7	10.5[a]	10.1[b]	10.1	9.9[b]	9.2[b]	8.9[b]
6	11.7	11.6	11.9	11.8	10.9	10.7	10.4[a]	10.6	10.6[a]	10.8	9.7[b]	10.1[a]
9	11.4	11.8	11.5	11.5	10.9	10.8[ac]	8.8[b]	8.7[bc]	10.5	11.8[c]	10.0[a]	10.2[ac]
11	11.4	11.0	10.9	10.8	10.4	9.8	9.0[b]	9.1[b]	10.1[a]	10.2	9.3[b]	9.3[a]
13	11.6	11.4	11.5	11.5	10.0	10.4	8.2[bd]	8.4[bc]	10.8	11.1	10.2[d]	10.1[c]

[a] Significantly different from reference at 5% level of significance
[b] Significantly different from reference at 1% level of significance
[c] Significant difference between W and HG at 5% level of significance
[d] Significant difference between W and HG at 1% level of significance
[e] 0 = odor acceptability; T = texture acceptability; F = flavor acceptability; All = overall acceptability.

musty, stale and metallic flavors while the HG fish had not. There was also a decrease in the proportion of tasters using the term sweet for the W fish from 0.58 on day 11 to 0.18 on day 13. These differences coincided with the acceptability rating for flavor on day 13 showing a downgrading for the W fish and suggested that the mushroom, cardboard, musty, stale and metallic flavors and the loss of sweetness described by the panel were disliked.

The shelf-life of orange roughy in ice can be considered as the period that has expired prior to the development of flavor aspects which are disliked by the panelists. In this trial the shelf-life of whole orange roughy in ice was therefore between 11 and 13 days while that of headed and gutted orange roughy in ice was between 13 and 16 days.

Microbiological results

Results from the microbiological analyses are presented in Fig. 1. On the surface of the whole fish, both the aerobic plate counts (APC) and sulfide producers showed an approximately log linear increase throughout the 16-day period. The APC increased from 3.1×10^3 CFU/cm^2 to 1.9×10^8 CFU/cm^2 while the sulfide producers increased from 2.7 CFU/cm^2 to 1.7×10^6 CFU/cm^2.

Throughout, sulfide producing bacteria constituted a very low proportion (often <0.1%) of the total aerobic flora. This suggested that the most common sulfide producer, *Alteromonas putrefaciens*, was not a major spoiler of orange roughy in this trial. It has been previously noted (Scott et al., 1984) that when sulfide producer counts form a low proportion of the total flora, crowding may occur making them difficult to count. This may explain the erratic nature of the curve for sulfide producers on the surface of the fish.

There were no consistent differences between the flesh counts of HG fish compared with those of the W fish. Day four flesh aerobic plate counts were lower ($p<0.025$) in the HG fish while at day 9 and day 16 they were lower ($p<0.01$) in the W fish. At day 16, however, the fish had already spoiled. At day 11 sulfide producer counts were lower ($p<0.05$) in the W fish. There were no significant differences at the other sampling periods. These results indicated that heading and gutting did not reduce the growth of bacteria in orange roughy flesh during ice storage. This did not correlate, however, with the differences observed in the flavor of the cooked fish.

Chemical results

Mean surface pH measurements during the storage period are shown in Fig. 2. At day 0 the pH of the flesh was 6.52. During storage there were no significant differences between the two treatments although there was a trend in both samples for the pH to increase, probably due to the production of volatile bases.

Dephosphorylation of inosine 5′-monophosphate (IMP) was

Table 3—Flavor of cooked orange roughy stored in ice

Flavor term	Days in Ice														
	4			6			9			11			13		
	R[b]	W	HG	R	W	HG	R	W	HG	R	W	HG	R	W	HG
Sweet	1.00[a]	0.67	0.60	0.76	0.76	0.59	0.79	0.50	0.50	0.68	0.58	0.47	0.88	0.18	0.53
Salty	0.27	0.20	0.20	0.41	0.35	0.18	0.36	0.29		0.32	0.32		0.24	0.18	0.24
Sour			0.27	0.18				0.29	0.21		0.26			0.24	0.29
Bitter		0.20	0.20		0.18	0.29		0.29	0.21	0.21		0.21		0.41	0.24
Lamby/ Meaty	0.27	0.20	0.27	0.29	0.29		0.43		0.29		0.21	0.26	0.41		0.24
Chicken						0.29	0.36		0.21	0.21	0.21		0.24		0.24
Shellfish				0.18			0.21								
Milky/ Creamy	0.67	0.67	0.67	0.65	0.53	0.35	0.64	0.36	0.57	0.42	0.47	0.37	0.53	0.41	0.47
Buttery	0.20			0.24	0.18		0.29		0.21	0.26		0.26	0.24		0.24
Caramel	0.20														
Nutty	0.27			0.29	0.24	0.24			0.29	0.32			0.24		
Neutral/ Bland	0.27	0.47	0.33		0.41	0.29		0.36	0.21	0.21	0.32	0.26		0.24	0.29
Mush-room								0.21						0.24	
Cardboard														0.18	
Potato									0.21						
Musty														0.24	
Flounder											0.21				
Stale														0.29	
Metallic								0.36						0.18	

[a] Figures are the proportion of the 12–20 panelists using a flavor term. Proportions were recorded if greater than 0.175.

[b] R = Reference; W = Whole; HG = Headed and gutted.

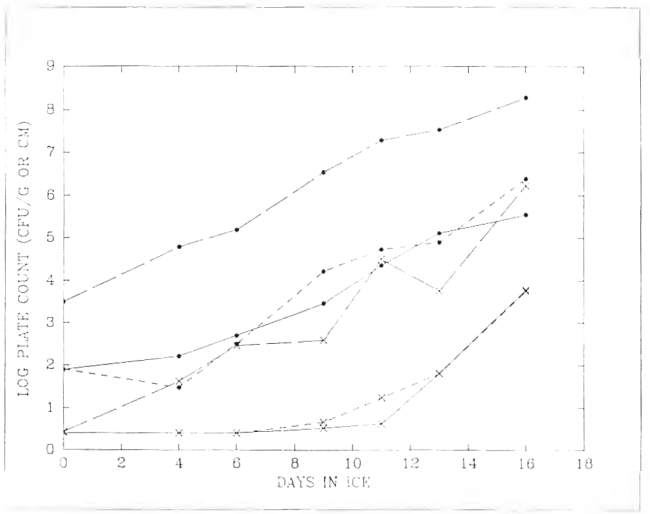

Fig. 1—Log plate counts of orange roughy: ○— —○ surface aerobic plate count (APC), whole; x— —x surface sulfide producers (SP), whole; ○——○ flesh APC, whole; ○----------○ flesh APC, headed and gutted (HG); x——x flesh SP, whole; x----------x flesh SP, HG.

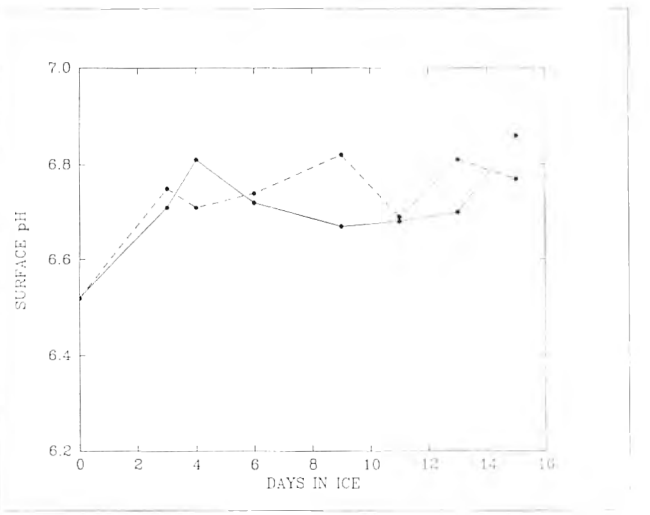

Fig. 2—Surface pH of orange roughy: ○----------○ whole, ○——○ headed and gutted. Plotted points represent the means of five sites on five fish.

approximately linear for the W fish during its shelf-life, while it plateaued in the later stages of the HG shelf-life (Fig. 3). The total pool of purine derivatives (adenosine 5′-triphosphate plus adenosine 5′-diphosphate plus adenosine 5′-monophosphate plus IMP plus inosine plus hypoxanthine) was the same for W and HG fish and showed a tendency to decrease from 7.7 μmol/g initially to 6.6 μmol/g after 13 days. The IMP levels dropped from 84% of the total pool of purine derivatives to 19% in the case of W fish and to 34% in HG fish during storage in ice over the 16 day period. There was no difference between IMP results for W and HG fish except on day 13 and day 16 when the HG fish had a higher IMP level ($p<0.05$). This correlated with sensory results which showed that the HG fish was still acceptable after 13 days.

The decrease in IMP was reflected by a corresponding increase in inosine levels throughout the trial (Fig. 4). These reached 57% and 49% for W and HG fish respectively at day 13, and then plateaued for HG and decreased further for W fish. There was a difference between inosine levels in W and HG fish on day 16 only, when HG fish had lower levels of inosine ($p<0.05$).

Hypoxanthine levels increased at a slow rate throughout the trial but never exceeded 20% of the total pool of purine derivatives. The hypoxanthine level has been shown to be a useful indicator of freshness in some species such as snapper (Boyd and Wilson, 1977) but according to our findings it is not appropriate for orange roughy.

The total combined amount of ATP, adenosine 5′-diphosphate (ADP) and adenosine monophosphate (AMP) remained constant throughout the trial at around 0.5–0.8 μmol/g fresh weight. There were no differences between W and HG samples.

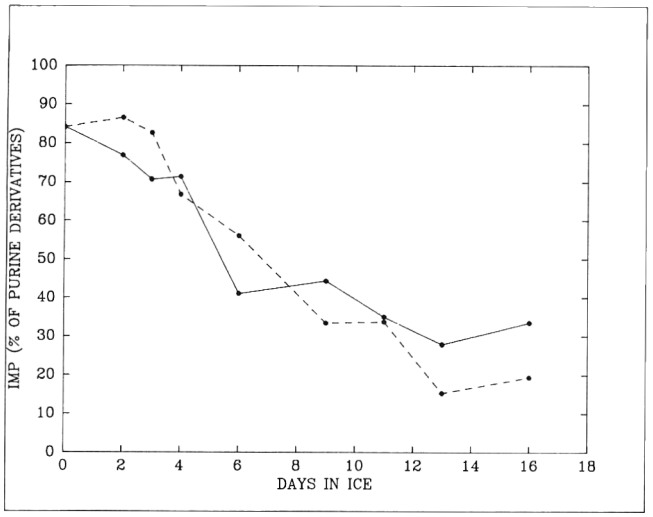

Fig. 3—Percentage of inosine 5'-monophosphate in the total pool of purine derivatives in orange roughy: ○----------○ whole, ○——○ headed and gutted.

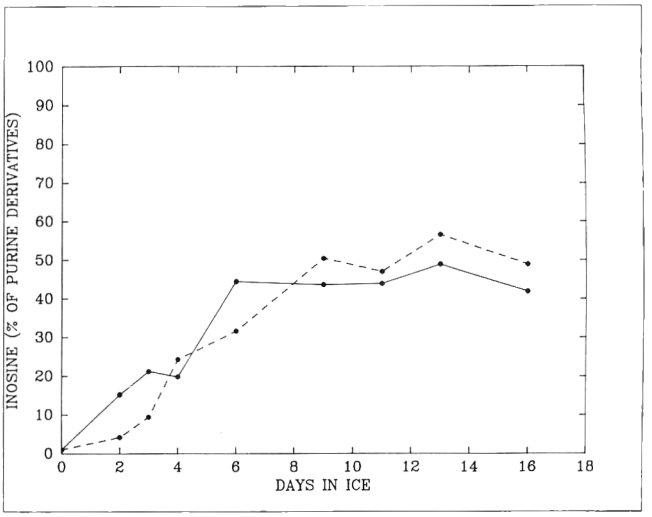

Fig. 4—Percentage of inosine in the total pool of purine derivatives in orange roughy: ○----------○ whole, ○——○ headed and gutted.

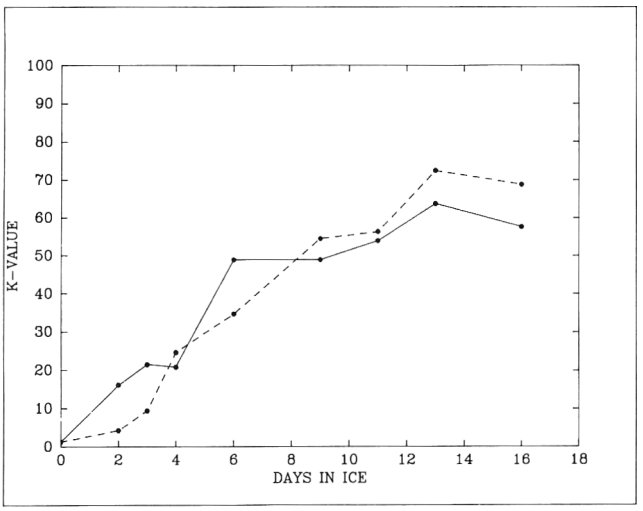

Fig. 5—K-value (%) of orange roughy: ○----------○ whole, ○——○ headed and gutted.

K value has been used by many workers for the objective assessment of freshness of fish (Aleman et al., 1982). The K value has been shown for some species to provide a better indication of early changes during storage in ice than the measurement of bacterial end products such as trimethylamine (Ryder et al., 1984). The K value for both W and HG fish increased at a constant rate from almost 0 to approximately 70 in 13 days (Fig. 5) There were not significant differences between W and HG fish over the first 13 days of storage in ice. On day 16 however, the W fish had a higher K value ($p<0.05$).

The K value provides a useful means of monitoring the loss of freshness of orange roughy during storage in ice and could be used as an objective measurement of freshness of orange roughy. Very fresh orange roughy will have a K value close to 0% while that at the end of its shelf-life will have a K value of 60% to 70%.

CONCLUSIONS

Taste panelists who were experienced in the assessment of fish described the flavor, texture, odor, and acceptability of orange roughy during storage in ice.

The shelf-life of whole orange roughy stored in ice was between 11 and 13 days with only a slight increase resulting from heading and gutting. This increase in shelf-life as determined by sensory evaluation was not reflected in microbiological results suggesting that the difference was due to reduced autolysis rather than to reduced microbial activity. This conclusion is supported by results of K value, IMP and inosine analyses which showed that there had been less advance of nucleotide breakdown in HG fish than in W fish beyond 13 days in ice. This may be attributed to the removal of the autolytic enzymes in the gut of the fish.

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A Rapid Ion-Pair HPLC Procedure for the Determination of Tyramine in Dairy Products

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ABSTRACT

A rapid high performance liquid chromatographic method for the determination of tyramine in dairy products was developed. The amine was extracted from foods with warm methanol, separated by ion-pair reversed phase chromatography and quantitatively determinated after o-phthalaldehyde post-column derivatization with a fluorescence detector. The calibration curve for tyramine in the range of 20–400 ng was linear and reproducible. The overall recovery rates were satisfactory.

INTRODUCTION

BIOLOGICALLY ACTIVE AMINES can be formed in food through the enzymatic decarboxylation of the corresponding amino acids. Of the biogenic amines, histamine and tyramine are the most interesting due to their physiological effects in man. Lüten (1983) gave an exhaustive review of the voluminous literature about the biogenic amines and their occurrence in certain foods. Many authors published on the presence of tyramine in cheese, wines, sauerkraut and other foods (Mayer and Pause, 1972; Pechaneck et al., 1983; Lovenberg, 1973; Van de Kerckhove, 1977).

The tyramine content in dairy products was studied in Spanish cheeses by Muñoz-Alcon et al. (1981) who found up to 1480 ppm whereas Pechaneck et al. (1983) found amounts between 51 and 696 ppm of tyramine in various cheese samples and Rivas Gonzalo et al. (1978) reviewed tyramine in dairy products. The need for a rapid and reliable technique for the determination of this amine is clear, since tyramine may be implicated in the appearance of migraine headaches. The enzyme, monoamine oxidase, oxidatively deaminates these amines and plays a major role in their degradation in man. Drugs known as monoamine oxidase inhibitors (MAOI) have been commonly prescribed for the treatment of mental depression and there have been numerous reports of hypertension crises of patients taking MAOI drugs (Blackwell and Mabbitt, 1965; Blackwell, 1963).

Various methods which have been described in the literature may be divided into two groups: (1) fluorometric determination after column cleanup procedures of the sample; (2) high-pressure liquid chromatography with various detection systems. The fluorometric method has been used for the determination of tyramine in many kind of foods by Muñoz-Alcon and Mariné Font (1979) and Jalon et al. (1983). After an appropriate extraction procedure, which may be followed by ion-exchange clean-up, the amine is detected after reaction with α-nitroso-β-naphthol as its fluorescence derivative. The lack of specificity of this method makes a qualitative identification necessary for those samples with apparently high contents of tyramine. Generally, a thin-layer chromatographic confirmation method is used.

During the last few years, analysis of biogenic amines in food by high pressure liquid chromatography procedures has increased. A wide variation of separation and detection techniques has been used and published. Hurst and Toomey (1981) described the determination of tyramine in chocolate using a Bondapak C_{18} column and two detection systems: ultraviolet absorbance at 254 nm and post-column o-phthalaldehyde (OPA) derivative formation and fluorescence detection. Koehler and Eitenmiller (1978) used reversed-phase HPLC of the sample extracts, previously purified by passing them through an ion-exchange column and UV-detection of the tyramine peak. Tyramine and other biogenic amines were determined by Hui and Taylor (1983), using HPLC and UV detection of the dansyl derivatives in their purified extracts. All these techniques require extensive sample preparation and often long analysis times.

Martín de Pozuelo et al. (1985) studied an ion-pair reversed-phase chromatographic determination with UV-detection, using the extraction method described by Jalon et al. (1983); however, the method was tedious, and the recoveries from spiked samples were low and variable.

The objective of this study was to develop a rapid and rather simple HPLC method for the qualitative and quantitative determination of tyramine in dairy products.

MATERIALS & METHODS

Standards

Tyramine hydrochloride salt from Fluka AG (Buchs, Switzerland) used as standard was prepared at an initial concentration of 50 mg in 100 mL of deionized distilled water and diluted with methanol to desired levels before use.

Samples

Experimental samples were commercial yogurt, two types of cheese, processed cheese and hard cheese and infant formulae.

Sample preparation and extraction

Two grams of sample were homogenized with approximately 40 mL of methanol in a Waring Blendor for 2 min. The homogenate was transferred to a 50 mL volumetric flask and placed in an oven at 60°C for 15 min. The flask was cooled with the aid of tap water, brought to volumn with methanol and filtered through Whatman #1 filter paper. An aliquot of 20 μL of the filtrate was injected into the chromatograph. Recoveries of tyramine in spiked samples were determined by adding 25–500 ppm tyramine to the dairy products.

High-pressure liquid chromatography

Chromatography was accomplished with a Waters liquid chromatograph equipped with a Model 6000 A pump, and a Model 730 Data Module. The chromatographic column was a Nova-Pak 5 μ C_{18} (end-capping) with radial compression (Waters Associates, Milford, MA). A guard-column packed with 37-50 μ Bondapak C_{18}/Corasil was used. A mobile phase of methanol:0.05M disodium hydrogen phosphate (15:85) containing heptane sulfonic acid as a counter-ion was prepared by adding 15 mL of PIC-B7-reagent (Waters Associates) to 1000 mL of mobile phase. The solvent was adjusted to pH 3.0 with 6M H_3PO_4. The flow rate was 1.5 mL/min. The fluorescent product was a post-column derivative produced in a three port mixing chamber (2.7×1.8×0.9 cm; 1.06×0.71×0.35 in.) purchased from Waters Associates. A second pump was used to pump the o-phthalaldehyde (OPA) solution into one port of the mixing chamber at a flow rate of

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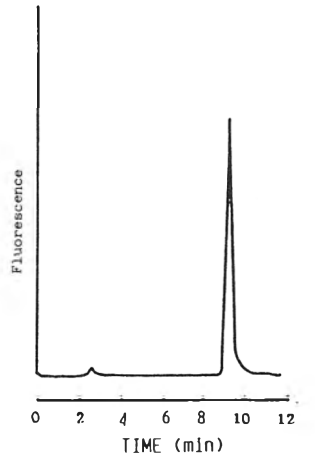

Fig. 1—Chromatogram of tyramine standard obtained by ion-pair HPLC after post-column o-phthalaldehyde derivatization.

1 mL/min. After mixing, the OPA-amine complex was carried into a reaction coil consisting of about 3m of 0.01 in. i.d. stainless-steel tubing, kept at a constant temperature at 40°C. The amines were detected and quantitatively determined as their OPA derivative with a fluorescence detector (Waters Fluorescence Detector, Model 420 with excitation wavelength at 338 nm and emission wavelength at 425 nm). The derivatizing reagent OPA (Fluka AG, Buchs, Switzerland) was prepared by dissolving 800 mg of OPA and 600 μL of mercaptoethanol in 10 mL of methanol; this mixture was made up to 1000 mL with 0.5M potassium borate buffer of pH 10.4.

RESULTS & DISCUSSION

IN THIS COMBINED METHOD, the chromatographic conditions to obtain an optimal separation of the tyramine peak from interfering peaks were studied. A Nova-Pak C_{18} column is specially designed to give good separations and no tailing of amines. The alkane sulfonic acids as counter ions have a widespread use in reversed-phase chromatography of amines; three different counter ions were tested: pentane, heptane, and octane sulfonic acid at pH 2.5, 3.0, and 4.0 and although the resulted retention times for tyramine were very similar, the conditions in which heptane sulfonic acid in the mobile phase was used, appeared to give the most symmetric peak at pH 3.0. However, the optimum pH for the OPA-mercaptoethanol derivatization of amines lies between 10.3 and 10.8 (Skaaden and Greibrokk, 1982). The difference between the pH of the mobile phase and the derivatizing agent is considerable; therefore, the ionic strength of the derivatizing buffer must be high enough to get an optimum pH value for the reaction. As can be seen in Fig. 1, a tyramine peak with no tailing is obtained under these conditions. Other biogenic amines as cadaverine, putrescine, and histamine were injected, but there was no interference with the tyramine peak. The reproducibility of the method is shown in Fig. 2 and the regression equation for this standard curve is:

$$\text{mm of peak height (at sensitivity} \times 16) = 0.12 \text{ ng of injected tyramine} - 0.50 \quad (r = 0.99)$$

With the proposed method up to 5 ng of tyramine/injection can be detected. The difference between the optimum pH of the chromatographic separation and the derivatization did not seem to affect reproducibility. The efficiency of the total extraction method was also performed with tyramine standard alone yielding the following regression equation:

$$\text{tyramine (extracted)} = 0.95 \text{ tyramine (added)} + 4.9 \quad (r = 1.02)$$

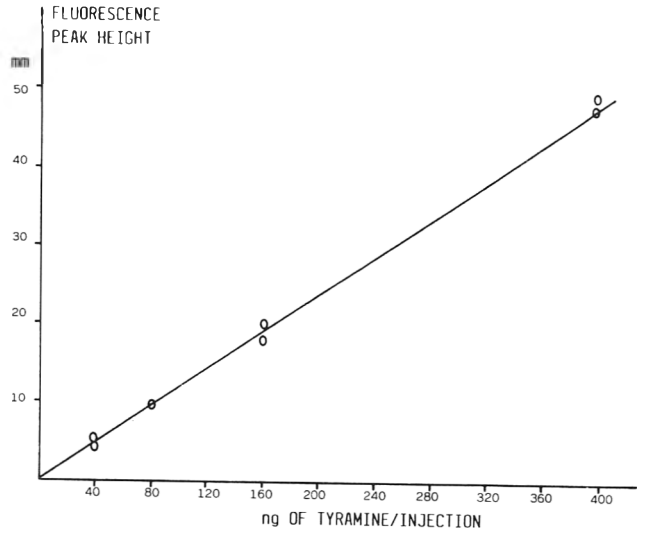

Fig. 2—Standard curve for tyramine, determined by ion-pair HPLC procedure. y (mm) = 0.12 ng tyramine + 0.5; r = 0.99.

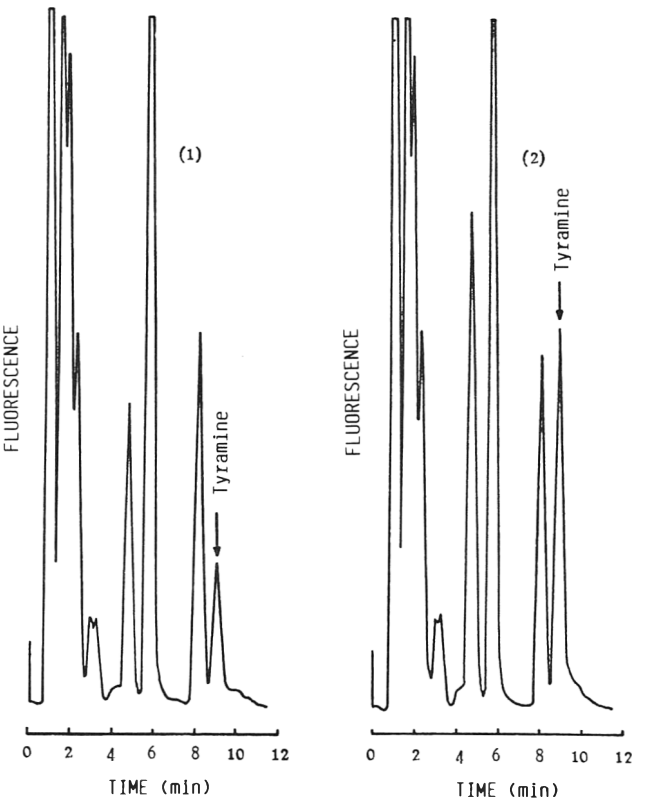

Fig. 3—Chromatogram of hard cheese with and without added tyramine. (1) 223 ppm tyramine; (2) with 500 ppm added tyramine.

The advantage of using OPA instead of dansylchloride is the selectivity of OPA-mercaptoethanol for primary amines, as was found by Himuro et al. (1983). The chemical reactions involved in the OPA derivatization are still not fully understood. The stability of the OPA-mercaptoethanol derivative is limited, so post-column derivatization was preferred, since the time between the formation of the derivative and its detection is very short. The method was applied to various kinds of dairy products; because of the expected difference in the extraction efficiency of the tyramine in the presence of food, some of the samples of infant formulae, yogurt and cheese were spiked with different amounts of tyramine to determine the recovery,

Table 1—Recoveries of tyramine added to different kind of dairy products and measured by HPLC after post-column o-phthalaldehyde derivatization

Sample	Background	Tyramine added	Tyramine found	Recovery[a]
	ppm	ppm	ppm	%
Infant formulae 1	—	25	27.2	110.0
Infant formulae 2	—	250	231.2	92.5
Infant formulae 3	—	500	481.0	96.2
Infant formulae 4	—	500	444.0	88.8
Infant formulae 5	—	500	444.0	88.8
Yogurt 1	—	50	50.0	100.0
Yogurt 2	—	100	104.0	104.0
Yogurt 3	—	500	510.0	102.0
Yogurt 4	—	500	508.0	101.6
Processed cheese 1	25	500	515.0	98.0
Processed cheese 2	27	500	414.0	77.4
Processed cheese 3	165	50	209.0	94.0
Processed cheese 3	165	100	259.0	94.0
Hard cheese 1	223	500	732.0	102.0
Hard cheese 1	223	500	714.0	98.2
Cottage cheese 1	—	470	460.0	98.0
Cottage cheese 2	—	470	484.0	103.0

[a] Average of duplicate injections.

efficiency, and sensitivity of the extraction, separation and detection method. With all these samples 2g were weighed and brought up to 50 mL with warm methanol. Every sample was injected twice. As can be seen in Table 1, only three samples of processed cheese and the sample of hard cheese contained tyramine in the unspiked product. The regression equation for the recovery is:

tyramine (measured) = 0.95 tyramine (spiked) + 1.5 ($r = 0.99$).

The recoveries yielded percentages between 77.4 and 110%, with an average recovery for infant formulae of 95.3%($CV = 0.09$), for yogurt of 102%($CV = 0.016$) and for cheese of 95.2%($CV = 0.084$).

Figure 3 illustrates a chromatogram of hard cheese which had 223 ppm tyramine and of the same cheese with 500 ppm added tyramine. There was no evidence for any special difficulty in the extraction of tyramine in these food products as can be seen from the recovery results. The detection limit of tyramine in this kind of food was at the low ppm range (1–5 ppm), which was sufficient for the toxicological significance of tyramine.

This HPLC method has several advantages for the analysis of tyramine in food, for it is a rapid and specific way to determine the tyramine content at rather sensitive levels.

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Heat-Induced Gelation of Individual Whey Proteins A Dynamic Rheological Study

MARIE PAULSSON, PER-OLOF HEGG, and HELGE B. CASTBERG

ABSTRACT

Heat-induced gelation of the bovine whey proteins [serum albumin (BSA), β-lactoglobulin (β-Lg) and α-lactalbumin (α-La)] has been studied individually and in mixture at different conditions by a dynamic rheological method. Values in the shear stiffness modulus (/G*/) appeared on heating at low protein concentration for BSA (~2%) and at intermediate concentration for β-Lg (~ 5%). α-La did not form a heat-induced gel of concentrations up to 20% (w/v). The ratio of viscous to elastic properties (loss factor) at maximum possible measuring temperature was below 0.07 for the BSA gels and 0.1–0.3 for the β-Lg gels. The temperature of gelation was highly dependent on pH. In mixture one protein could not be exchange for another without changing the gelation behavior of the mixture.

INTRODUCTION

AN IMPORTANT technological property of a protein is the ability of gel formation (Kinsella, 1976, 1983).

The rheological properties of milk and crude milk protein concentrates are very complex in nature. Generally, temperature, protein concentration and physical state of the dispersed phase determine the rheological behavior. Viscosity characteristics and conditions for gel formation in milk, milk protein concentrates and whey protein concentrates have been investigated by a variety of methods (Bertsch and Cerf, 1983; Buckingham, 1978; Fernández-Martín, 1972; Kalab et al., 1970; Hermansson, 1975). Studies on gel characteristics were done using instruments based on empirical data like tube viscometers, Höppler viscosimeter and penetrometers.

Few studies have been published on heat-induced gelation of individual whey proteins. Such studies on individual proteins in well defined systems seem appropriate in order to obtain a better understanding of gel formation of protein mixtures in different environments. Thermally induced gels of serum albumin and β-lactoglobulin have been examined at various salt concentrations and pH-values by Hegg (1982). Serum albumin was found to have better gelling properties than β-lactoglobulin. However, there were two limitations to the study: an unfavorably high heating rate for gelation and the measurement of gel formation by a destructive and empirical centrifugation technique.

The dynamic rheological technique (Mitchell, 1980) allows the study of gel formation without affecting the process. This technique was used earlier to study the gelation of rennet milk (Tokita et al., 1983) and to characterize the rheological properties of ovalbumin (van Kleef et al., 1978). An instrument to measure continuously the viscoelastic properties of a gel by this technique has recently been developed in our laboratory (Bohlin et al., 1984). In the present investigation this instrument, which gives total shear stiffness, viscous and elastic properties in physical units, is used to examine the heat-induced gelation properties of the three main bovine whey proteins — serum albumin, β-lactoglobulin, and α-lactalbumin — at conditions relevant in food technology.

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MATERIALS & METHODS

Materials

Bovine serum albumin (BSA) (A-0281, lot no 63F-9350), β-lactoglobulin (β-Lg) (L-6879, lot no 111F-8025), and α-lactalbumin (α-La) (L-6010, lot no 52F-8075) were obtained from Sigma Chemical Co. α-La contained less than 0.3 moles of calcium per mol of protein. β-Lg was desalted by dialysis against glass distilled water and freeze dried to constant weight. BSA was saltfree and essentially free of fatty acids as checked by differential scanning calorimetry (DSC) (Gumpen et al., 1979). No contamination of β-Lg or BSA with other proteins could be detected by SDS-poly-acrylamide gel electrophoresis or by DSC, while α-La was > 90% pure, mainly contaminated by β-Lg.

Simulated milk ultrafiltrate (SMUF) was prepared according to Jenness and Koops (1962). The salts were purchased from Merck AG and were of analytical grade.

Methods

Heat-induced gelation of the whey proteins, BSA, β-Lg and α-La was measured by a dynamic rheological method with the instrument recently described by Bohlin et al. (1984). This method gives, without affecting the gel formation process, the viscoelastic properties of a protein gel as a function of temperature at a fixed frequency (0.5 Hz). The instrument records both the complex modulus (/G*/) and the phase shift (δ). The complex modulus gives a measure of the total shear stiffness of the protein gel and the phase shift measures the relative contribution of elastic and viscous flow in the gel. For an ideal viscous liquid the phase shift is 90°, whereas for an ideal elastic body the phase shift is 0°. A desk top computer controls the measuring process and calculates storage modulus (G'), loss modulus (G") and loss factor (tan δ, ratio of viscous to elastic properties).

The proteins were dissolved in 1% NaCl or SMUF and adjusted to appropriate pH value with 1M HCl or 1M NaOH. A 2 mL sample of protein solution was pipetted into the measuring cell of the instrument. The protein solution was heated from 30°C to 95°C at a rate of 1°C/min and the various parameters were recorded every minute. The highest amplification was used and the shear strain amplitude, γ, was constant (0.15).

RESULTS & DISCUSSION

Gelation at different protein concentrations

A minimum concentration of protein is required for formation of heat-induced gels. Substantial concentrations are frequently used in studies reported on protein gelation (Clark et al., 1981). Even if heat-induced gelation of globular proteins is dependent on the experimental condition used (Hegg, 1982), determination of the lowest protein concentration for gel formation might be used as a criterion for, or as a classification of, the ability of specific proteins to gel.

Heat-induced gelation at increasing concentrations of the various proteins at pH 6.6 and 1% NaCl is shown in Fig. 1. The minimum concentration needed to obtain a gel was quite different for the three proteins; for BSA 1% (Fig. 1a), and for β-Lg around 2% (Fig. 1b). α-La did not gel under these conditions even up to a protein concentration of 20% (w/v). The temperature at which gelation started decreased with increasing protein concentration whereas the slope of the complex modulus, /G*/, versus temperature increased. The initiation temperature of gelation differed between the proteins. Gelation of BSA started in the range 70–90°C, while 75–80°C was observed for β-Lg. It is reasonable to assume that gelation is the result of major conformational changes induced by the thermal

Table 1—The phase shift (δ) and loss factor (tan δ) of β-lactoglobulin and bovine serum albumin gels (1% NaCl, pH 6.6) at different protein concentrations

β-Lactoglobulin				Bovine serum albumin			
Concentration (%)	δ degrees	tan δ	Temperature[a] (°C)	Concentration (%)	δ degrees	tan δ	Temperature[a] (°C)
9	17.81	0.32	80	5	3.96	0.07	71
5	10.91	0.19	86	2	0.00	0.00	89
3	6.07	0.11	95	1	0.43	0.01	95

[a] Values are given for the temperature at the end of the gelation process.

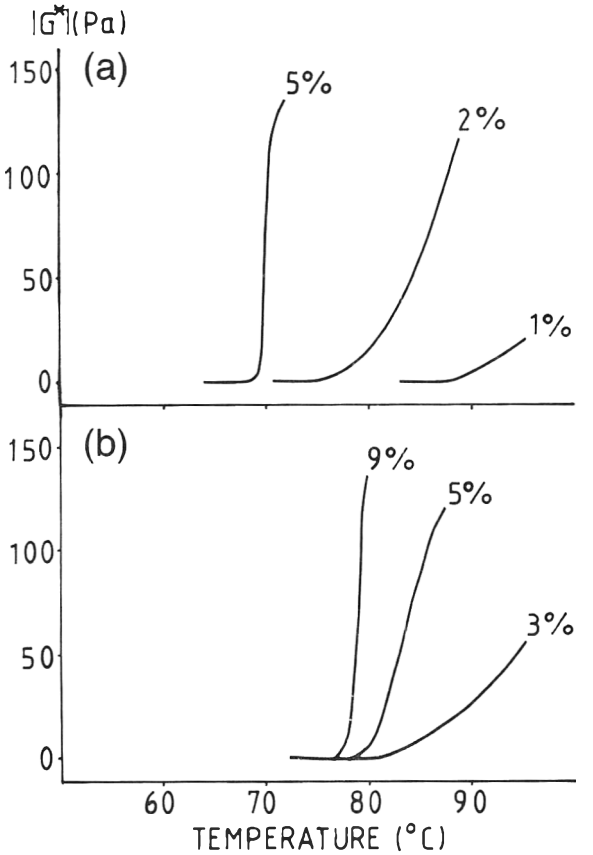

Fig. 1—Total shear stiffness, /G/, of whey protein gels at pH 6.6 in 1% NaCl as a function of temperature; heating rate, 1°C/min. (a) Bovine serum albumin at different concentrations; (b) β-Lactoglobulin at different concentrations.*

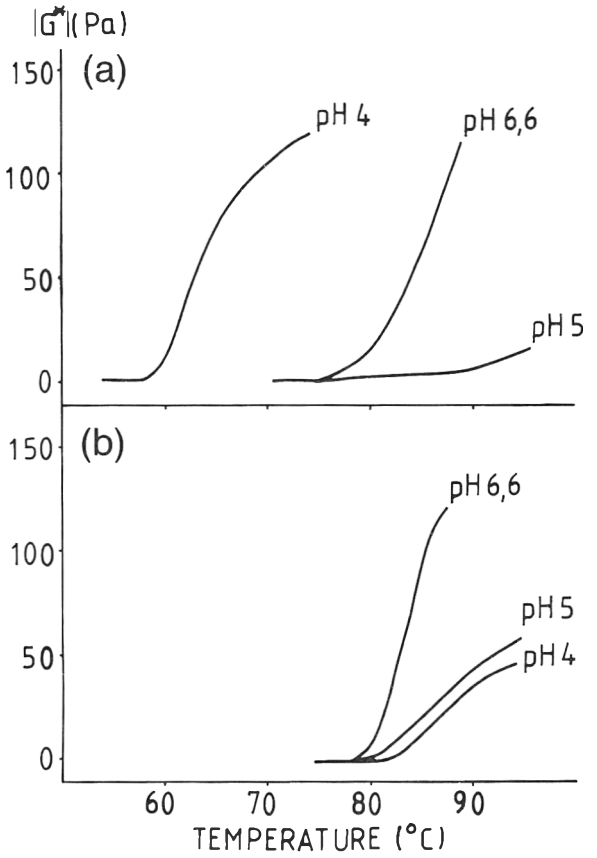

Fig. 2—Total shear stiffness, /G/, of whey protein gels in 1% NaCl as a function of temperature; heating rate, 1°C/min. (a) Bovine serum albumin (2% w/v) at different pH-values; (b) β-Lactoglobulin (5% w/v) at different pH-values.*

denaturation of the proteins. Correlation between gelation and denaturation of a protein is, however, difficult to elucidate since initiation of gelation was concentration dependent (Fig. 1) and influenced by ionic strength, pH, etc. (Hegg, 1978). Ionic strength might also be an important parameter in protein denaturation (Gumpen et al., 1979) and consequently an accurate comparison between initiation of gelation and denaturation requires detailed knowledge for every specific condition.

According to the minimal concentration of protein required for gel formation at pH 6.6, and 1% NaCl, BSA might be characterized as having good, β-Lg intermediate and α-La poor thermal gelation properties.

Final values of the shear stiffness modulus (/G*/) are not given in this investigation. It is obvious however, that whey protein gels formed by heat were stiffer than gels of renneted milk, where values of /G*/ up to 80 Pa were found (Bohlin et al., 1984). Beside complex modulus the instrument used gave information on viscous (loss) modulus and elastic (storage) modulus. The phase shift normally decreased when temperature increased, i.e. the gels became increasingly elastic. The phase shift, δ, and tan δ, (a measure of the ratio between the viscous and elastic character of the gel) are given for BSA and β-Lg at the end point temperature of the curves obtained at the different concentrations (Table 1). BSA had a phase shift near 0° and the gels thus seem "ideally" elastic. β-Lg had phase shifts between 0° and 90° (viscoelastic gels) at all temperatures examined. An increase in the concentration thus resulted in a more viscous gel, possibly due to gelation at a lower temperature. It should be pointed out that tan δ could not be compared at equal temperatures for the different concentrations.

Gelation at acidic pH values

The isoelectric points of the whey proteins are very close; BSA at pH 5.1 (Fox, 1982), β-Lg at pH 5.2 (McKenzie, 1971), and α-La at pH 4.2–4.5 (Fox, 1982). The three proteins will thus have a slight negative net charge at pH 6.6.

The effect of the protein net charge on heat-induced gelation was studied at acidic pH values at a fixed protein concentration for each protein. The lowest concentration required to yield a gel strength of minimum 100 Pa at temperatures below 95°C was selected. The protein concentrations used were 2% for BSA and 5% for β-Lg.

Figure 2 shows the complex modulus versus temperature at pH 4.0, 5.0, and 6.6. The gel formation of BSA at pH 4.0 (Fig. 2a) started at a low temperature (60°C); however, at pH 5.0 gel formation began at 85°C. The gel strength at pH 5.0 was very low and did not increase very much between 85° and 95°C. Evidently, gel formation can be controlled by regulating the pH. The results are in accordance with phase diagrams on gelling behavior reported earlier (Hegg, 1982) when gelation

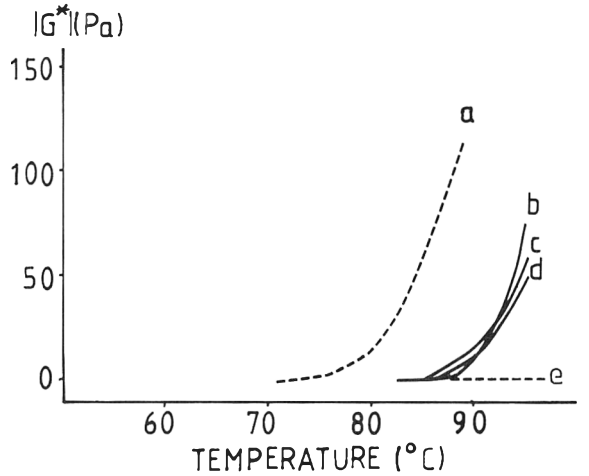

Fig. 3—Total shear stiffness, |G|, of bovine serum albumin and α-lactalbumin as a function of temperature. Proteins were dissolved in 1% NaCl at pH 6.6; heating rate, 1°C/min. (a) Bovine serum albumin at a concentration of 2% (w/v); (b) A mixture of bovine serum albumin (2% w/v) and α-lactalbumin (5% w/v); (c) A mixture of bovine serum albumin (2% w/v) and α-lactalbumin (5% w/v); (d) A mixture of bovine serum albumin (2% w/v) and α-lactalbumin (3% w/v); (e) α-Lactalbumin up to 20% (w/v).*

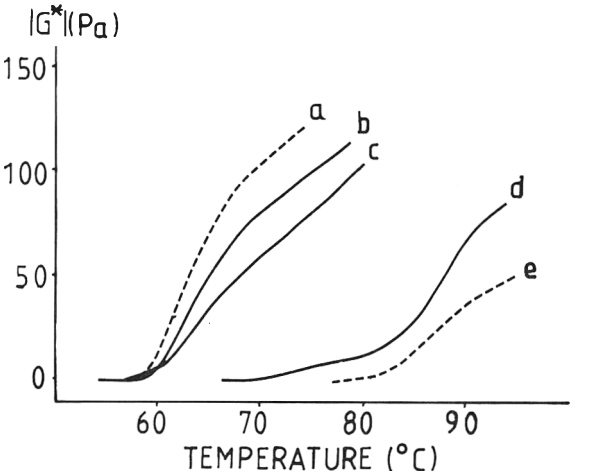

Fig. 4—Total shear stiffness, |G|, of bovine serum albumin and β-lactoglobulin as a function of temperature. Proteins were dissolved in 1% NaCl at pH 4; heating rate, 1°C/min. (a) Bovine serum albumin at a concentration of 2% (w/v); (b) A mixture of bovine serum albumin (2% w/v) and β-lactoglobulin (2% w/v); (c) A mixture of bovine serum albumin (2% w/v) and β-lactoglobulin (5% w/v); (d) A mixture of bovine serum albumin (1% w/v) and β-lactoglobulin (1% w/v); (e) β-Lactoglobulin at a concentration of 5% (w/v).*

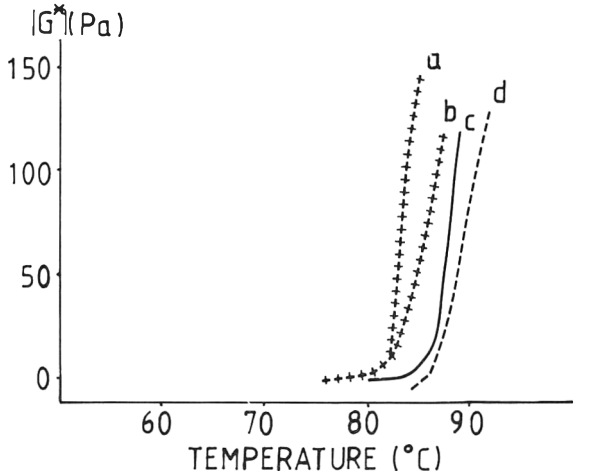

Fig. 5—Total shear stiffness, |G|, of a whey protein mixture as a function of temperature. Proteins were dissolved in SMUF at pH 6.6; heating rate, 1°C/min. (a) β-Lactoglobulin at a concentration of 5% (w/v); (b) Bovine serum albumin at a concentration of 2% (w/v); (c) A mixture of 1.2% (w/v) α-lactalbumin + 3.2% (w/v) β-Lactoglobulin + 0.4% (w/v) bovine serum albumin; d) β-Lactoglobulin at a concentration of 3.2% (w/v).*

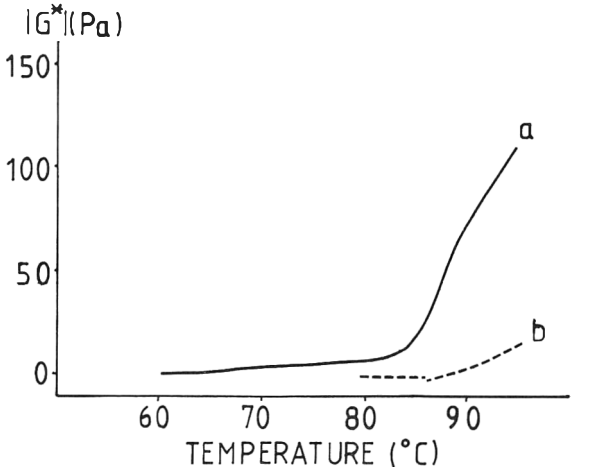

Fig. 6—Total shear stiffness, |G|, of a whey protein mixture as a function of temperature. Proteins were dissolved in SMUF at pH 4; heating rate, 1°C/min. (a) A mixture of 1.2% (w/v) α-lactalbumin + 3.2% (w/v) β-lactoglobulin + 0.4% (w/v) bovine serum albumin; (b) β-Lactoglobulin 3.2% (w/v).*

was not found at the isoelectric point (pH 5.0). The probability for gel formation should be the lowest at the isoelectric point for electrostatic reasons and increase with increasing positive or negative net charge.

The gelation temperature of β-Lg was less influenced by pH than that of BSA (Fig. 2b). In contrast to BSA, β-Lg showed moderate gel texture even at the isoelectric point. This discrepancy, compared to earlier findings (Hegg, 1982), might be explained, partly, by the different heating rates used. A high heating rate seemed unfavorable for heat induced gelation (Hegg, 1978). The similar pattern observed for β-Lg at pH 4.0 and pH 5.0 might reflect the comparably poor gelling ability of this protein at low pH values.

α-La did not gel at concentrations up to 20% (w/v) at pH 5.0. At pH 4.0 the protein precipitated at room temperature precluding measurements.

The studies on the effect of pH on gel formation were performed only at one protein concentration. It is reasonable to assume that the influence of pH would diminish when the protein concentration is increased. The ratio between viscous and elastic component for BSA was close to 0 and that for β-Lg around 0.2 at all pH values examined.

Gelation of protein mixtures

It can be concluded that heat-induced gelation of the whey proteins can be altered by changes in protein concentration and pH. Another way to influence gel formation may be by mixing individual proteins since the presence of other proteins might modify the gel characteristics observed in a single component system. From the results obtained in Fig. 1 and 2, several sets of conditions were selected to study mixed protein systems.

BSA and α-La at pH 6.6

At pH 6.6 and 1% NaCl BSA showed excellent gelling properties while α-La did not gel. It was therefore of interest

to determine whether α-La influenced the gelation properties of BSA at this pH. A mixture of 2% BSA and various concentrations of α-La (Fig. 3, curves b-d) was compared with pure BSA (curve a) and pure α-La (curve e). The start of gelation of the mixture shifted to a higher temperature compared with BSA alone. Evidently, α-La was not only a poor gelling protein but, in the sense of increasing the gelation temperature, also make a good gelling protein less inclined to gel. Notably, the formation of a BSA gel framework was disturbed as much by a 1% addition of α-La as by a 5% addition.

BSA and β-Lg at pH 4

BSA and β-Lg have widely different gelation temperatures and gel strengths at pH 4.0. A mixture of 2% BSA and various concentrations of β-Lg was compared with pure β-Lg (Fig. 4, curve e) and pure BSA (curve a). Increasing concentrations of β-Lg (curves b and c) gradually shifted the BSA curve towards higher temperatures. The gelation curves of the mixed proteins were not a resultant of an additive effect of the proteins. Instead BSA clearly dominated the gelation process and its gelation characteristics were largely preserved. However, the addition of 5% β-Lg slightly increased the viscous character of the BSA gel shown by a tan δ of 0.09 at 80°C (cf. Table 1).

A mixture of 1% BSA and 1% β-Lg (Fig. 4, curve d) further showed that the proteins were not mutually interchangeable without changing the gel. The gelation ability of BSA was significantly strengthened by β-Lg, probably due to the fact that the protein concentration of the two separate proteins (1%) is close to (BSA), or below (β-Lg), the limit for gel formation.

Mixtures of whey proteins in SMUF

The gelation behavior of pure BSA (2% w/v) and pure β-Lg (5% w/v) in SMUF (pH 6.6) are shown in Fig. 5 (curves a and b). The start of gelation for these proteins in SMUF occurred at higher temperatures than in 1% NaCl (cf. Fig. 2a,b), whereas no gel formed for α-La. The different behavior in NaCl and SMUF is probably explained by the different ionic composition and ionic strength. The increase in gelation temperature in SMUF was a general phenomenon, found both at different protein concentrations and pH values.

The gel characteristics of a mixture of the three whey proteins at ratios appropriate to whey are described by curve c (Fig. 5). The total protein concentration was 4.8% (pH 6.6 in SMUF). Neither α-La nor BSA was individually capable of gelling at the concentrations of the mixture. The gelation of 3.2% pure β-Lg is given by curve d. The gel formed from the mixture of the whey proteins is clearly dominated by β-Lg; thus it seems possible to realize the gelation pattern of a protein mixture by knowledge of the gelation behavior of the individual proteins at appropriate conditions. It can be inferred from the appearance of curves a and b, that a higher total mixture concentration would marginally influence the gelation pattern.

Figure 6 shows the gel formation of the whey protein mixture at pH 4 (curve a). At the concentration of the mixture, β-Lg as such was at the limit to form a heat-induced gel (curve b), while neither α-La nor BSA formed gels at this pH. It was concluded earlier, that additive or even synergistic effects on heat-induced gelation, could be achieved when proteins were mixed at concentrations below or close to the gelation limit. Here α-La and/or BSA markedly contributed to the gelation pattern of the whey mixture. The ratio of viscous to elastic properties (tan δ) for the whey mixture was 0.2, however, indicating that the characteristics of the gel formed were still determined by β-Lg (cf. Table 1).

CONCLUSIONS

THE PRESENTED RESULTS on viscoelastic properties of the individual whey proteins are unique since the instrument used does not affect the gelation process. Furthermore, the registered stiffness modulus could be separated into an elastic and a viscous component measured in physical units. The various whey proteins were characterized as BSA having good, β-Lg intermediate and α-La poor thermal gelation properties. The BSA gels were purely elastic, while the gels of β-Lg had viscous elements. Thermal gelation of the individual whey proteins could be controlled by changes in protein concentration, pH and ionic strength.

In mixture, the influence on heat-induced gelation of the whey proteins at equal concentrations decreased in the order BSA, β-Lg, α-La, while a protein at high concentration usually determined the gelation behavior. At acidic pH-values BSA dominated the gel characteristics. α-La was a poor gelling protein and had also negative influence on the gelation properties of other proteins. These conclusions illustrate the importance of comprehending the gelation of individual proteins to be able to understand the gelation pattern of a protein mixture. However, it is difficult to predict the gelation pattern when the whey proteins are mixed below or at their individual critical gel concentrations. In these cases the gelation behavior seems to be governed by synergistic effects.

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Compositional Factors That Affect the Emulsifying and Foaming Properties of Whey Protein Concentrates

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ABSTRACT

An emulsion containing 30% fat was used to study emulsifying and foaming properties of commercial whey protein concentrates. Residual lipids, both total and phospholipids, inhibited foaming of dilute aqueous solutions of whey protein concentrates, whereas in aerated emulsions residual lipids were positively correlated with foaming. Under both test conditions the ash content was positively correlated with good and moderate foaming properties. Among the compositional factors which best predicted foaming of emulsions were sulfhydryls. Emulsions which contained insoluble whey proteins were highly stable but air incorporation was poor. When soluble whey proteins were utilized to stabilize emulsions, serum separation occurred more readily but did not correlate with good foaming properties.

INTRODUCTION

COMMERCIALLY AVAILABLE whey products, described as whey protein concentrates (WPC), are prepared by physically or chemically separating the protein from the lactose and minerals in the raw whey. Food products for which WPC are often mentioned as having potential utility are emulsions and foams. A number of workers have studied the factors that are important for proteins to function in simple foams (Hansen and Black, 1972; Jelen, 1973; Richert et al., 1974) and emulsions (Kuehler and Stine, 1974; Pearce and Kinsella, 1978; Yamauchi et al., 1980). There have been fewer studies of aerated emulsions (Nash and Brickman, 1972; Petrowski, 1976; Min and Thomas, 1977).

Whey inherently shows variability, and it is difficult to standardize the functional properties of concentrates derived from it. One of the main problems that has inhibited the use of WPC in food has been the difficulty of producing products with constant functional properties (Melachouris, 1984; Schmidt et al., 1984). While these proteins have been shown to be satisfactory by many workers in simple routine screening tests, experience in more complex systems has demonstrated a lack of consistency (Harper, 1984).

The purpose of the present study was to gain a better understanding of the chemical and physical properties of WPC that affect their performance in aerated emulsions.

MATERIALS & METHODS

Whey protein concentrates

A diafiltration WPC was obtained from Westerville Creamery (Covington, OH). Products produced by a combination of lactose crystallization and diafiltration both unneutralized and neutralized, were obtained from Foremost-McKesson (San Francisco, CA). Metaphosphate complexed WPCs in the acid, Na-neutralized and Ca-neutralized forms were obtained from Borden, Inc., Elgin, IL.

A control WPC was prepared from fresh milk separated at 37°C. The skim was adjusted to pH 4.6 by gradual addition of 1M HCl. The whey was concentrated by rotary evaporation at 40°C to 35% total solids and dialyzed for 100 hr against several changes of distilled water at 4°C. The dialyzate was freeze-dried and contained 94.8% protein.

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Chemical analysis

Protein was determined by the Kjeldahl procedure of Scales and Harrison (1920). Moisture was determined at 100 + 2°C for 5 hr according to the standard procedure for rennet caseins (BSI, 1962).

Lactose was determined on WPCs that were dissolved in distilled water to give 1 to 3% solutions by the HPLC procedure of Marsili et al. (1981). To determine ash, samples were charred using a flame and then ignited overnight in a muffle furnace at 550°C. Use of acid was necessary because in most cases the procedure failed to yield carbon-free ash. Concentrated HCl was added, evaporated to dryness on a steam-bath and the samples ashed overnight at 550°C. Concentrated HNO_3 was used in a similar fashion until a constant sample weight was obtained.

Calcium was determined by atomic absorption with a Perkin-Elmer Model 340. Phosphorus was determined by the method of Morrison (1964).

Lipids were determined by the AOAC Method (AOAC, 1975). Phospholipid was determined on the extracted lipids by multiplying the phosphorus content (Morrison, 1964) by a factor of 25.5 (McDowell, 1958).

Total sulfhydryls were determined as described by Patrick and Swaisgood (1976). Free and reactive sulfhydryls were determined by the method of Hardham (1981).

Protein fractionation

To increase the number of samples available and the range of concentration of constituents, the WPCs were fractioned into soluble and insoluble fractions. The pH of 5% protein dispersion was adjusted to 4.6 with 1N NaOH or HCl. After equilibration for 1 hr at room temperature (RT) the pH was readjusted to 4.6, if necessary, and the samples centrifuged at 12,000 × *g* for 30 min at 5°C. The supernatant and sediment were freeze-dried and analyzed along with the total WPC.

Functional properties

The foaming test of Aguilera and Kosikowski (1978) was utilized to determine the ability of the WPC to form and maintain a foam structure in water. The model whipped topping system, described by Harper et al. (1980), was utilized to determine the ability of the WPC to form a high fat foam. The composition of the model topping is given in Table 1. The hematocrit centrifugal method of McDermott et al. (1981) was employed to determine the stability of the topping before foaming. The results were expressed as Emulsion Volume Index (EVI), where:

$$EVI = \frac{(\text{length of emulsion phase/total length of column}) \times 100}{\%\ \text{fat w/w in emulsion}/0.9}$$

Table 1—Composition of model whipped topping

Formulation	Percent
Coconut Oil	30
Protein[a]	6
Sugar	7
Stabilizers (Carrageenan, Guar Gum, CMC in ratio of 1:1:3)	0.2
Emulsifiers (Tween 60, Span 60)	0.05
Water to make to	100

[a] Sodium caseinate is used as reference protein.

Table 2—Composition of commercial WPC and their soluble and insoluble fractions

Type of WPC	% Protein	% Lactose	% Ash	% Phosphorous	Calcium mg/100g	% Fat	Phospholipid as % of fat
WHOLE WPC							
Na-metaphosphate	59.3	15.75	12.18	3.30	13.9	6.76	13.9
Ca-metaphosphate	57.6	20.29	11.78	3.03	2180.0	6.81	15.3
Acid metaphosphate	55.3	25.89	7.58	3.53	19.3	6.19	6.2
Diafiltration	89.3	1.08	0.98	0.26	118.6	5.08	33.0
K-electrodialysis	35.2	63.56	2.91	0.48	256.0	3.92	16.4
Electrodialysis, (Unneutralized)	36.2	53.85	1.67	0.46	251.0	5.29	13.7
SUPERNATANT							
Na-metaphosphate	34.1	32.16	16.76	3.93	17.2	6.01	22.1
Ca-metaphosphate	54.6	24.78	8.33	1.78	1426.0	3.04	15.3
Acid metaphosphate	39.7	32.96	15.68	4.54	14.9	0.48	23.8
Diafiltration	88.6	1.53	1.42	0.13	160.0	0.57	31.9
K-electrodialysis	30.3	61.68	3.20	0.48	280.0	3.15	19.4
Electrodialysis, (Unneutralized)	31.2	63.00	1.67	0.43	259.0	2.33	15.6
SEDIMENT							
Na-metaphosphate	67.1	8.04	8.68	2.54	16.9	6.24	11.9
Ca-metaphosphate	51.8	7.22	18.21	5.27	3669.0	12.50	14.3
Acid metaphosphate	65.5	9.27	5.86	1.87	22.0	11.67	5.1
Diafiltration	82.0	1.02	0.85	0.36	36.1	9.86	31.2
K-electrodialysis	53.4	33.13	1.96	0.33	183.0	4.81	13.0
Electrodialysis, (Unneutralized)	47.2	34.97	1.50	0.38	231.0	11.71	10.5

Table 3—Sulfhydryl concentrations (μM/g protein) of commercial WPC and their soluble and insoluble fractions

Type of WPC	Total SH	Free SH	Reactive SH
WHOLE WPC			
Na-metaphosphate	280.4	25.2	21.4
Ca-metaphosphate	281.5	23.1	17.0
Acid metaphosphate	283.9	24.7	13.8
Diafiltration	241.0	29.9	25.6
K-electrodialysis	203.2	12.2	4.6
Electrodialysis, (Unneutralized)	205.7	15.5	14.6
SUPERNATANT			
Na-metaphosphate	202.0	19.8	16.6
Ca-metaphosphate	271.1	26.5	22.0
Acid metaphosphate	233.4	37.5	28.5
Diafiltration	243.7	36.2	25.2
K-electrodialysis	185.9	14.6	6.6
Electrodialysis, (Unneutralized)	182.5	18.8	11.2
SEDIMENT			
Na-metaphosphate	299.5	27.1	18.7
Ca-metaphosphate	287.0	14.4	7.1
Acid metaphosphate	288.3	13.9	6.3
Diafiltration	230.8	14.5	9.4
K-electrodialysis	245.5	5.4	3.3
Electrodialysis, (Unneutralized)	240.7	7.7	6.2

Table 4—Foaming and emulsifying properties of commercial WPCs and their soluble and insoluble fractions

Type of WPC	Foam capacity	Foam stability[a]	Max overrun	Emulsion rating[b] (%)
WHOLE WPC				
Na-metaphosphate	90	76	247.8	86.06
Ca-metaphosphate	80	36	264.2	91.40
Acid metaphosphate	61	40	256.5	81.11
Diafiltration	80	73	101.8	87.24
K-electrodialysis	98	70	226.0	83.20
Electrodialysis, (unneutralized)	110	90	198.4	86.22
SUPERNATANT				
Na-metaphosphate	133	102	285.0	56.28
Ca-metaphosphate	128	102	330.4	67.26
Acid metaphosphate	174	168	252.5	50.26
Diafiltration	154	148	223.7	59.93
K-electrodialysis	124	111	218.2	61.65
Electrodialysis, (unneutralized)	165	154	191.1	65.48
SEDIMENT				
Na-metaphosphate	46	23	118.2	87.64
Ca-metaphosphate	62	16	55.2	99.31
Acid metaphosphate	58	12	203.8	91.14
Diafiltration	54	50	53.3	98.51
K-electrodialysis	50	40	144.1	97.65
Electrodialysis, (unneutralized)	88	86	126.5	94.79

[a] Foam stability 15 min after air incorporation.
[b] Emulsion Volume Index after 40 min of centrifugation.

EVI provides a measure of the interglobular volume and serves as an index of the resistance of the emulsions to compression during specific experimental conditions. From this value the emulsion rating was determined. The emulsion rating is the percentage of the experimental EVI from a theoretical EVI assuming no oil separation occurred.

Statistical models

All analyses were performed in triplicate and the standard deviation was less than 5% on all assays performed. First-order general linear regression models for different responses were used to provide a description of the relationships between compositions of WPC and their functional characteristics. Factors that contributed to functionality were statistically selected by submitting the compositional data of WPC as independent variables with the functional characteristic being studied as the response to a stepwise multiple regression analysis. The final set of variables tested in the model contained those which showed correlation with the response but which were not highly correlated with each other. In addition, possible interaction terms of these variables were chosen on the same basis.

RESULTS & DISCUSSION

THE COMPOSITION of the commercial WPCs and the derived supernatant and sediment fractions are listed in Table 2. Data for the content of total, free and reactive sulfhydryls are presented in Table 3. The functional properties of the WPCs are listed in Table 4.

Foam capacity and stability

In the simple foaming test a single compositional variable which explained 72% and 77% foaming capacity (FC) and foaming stability (FS), respectively, turned out to be disulfide concentration. Figure 1 shows the goodness of fit for the best three-variable model predicting FC. This model indicated that ash content was positively correlated, whereas, phospholipid and disulfide concentration were both negatively correlated with

Table 5—Stepwise multiple regression analysis relating compositional variables to overrun of whipped topping stabilized with whey protein concentrates

Protein Source	R^2	Variable	β-Coefficient	Prob. >F
Whey protein concentrate	0.771	Intercept	136.256	
		Ash	0.105	0.0001
	0.975	Intercept	220.888	
		Free Sulfhydryls[a]	−0.708	0.0001
		Ash	0.116	0.0001
	0.989	Intercept	135.558	
		Ash	0.093	0.0001
		Fat	0.081	0.0006
		Reactive Sulfhydryls[a]	−0.449	0.0001
Supernatant fractions	0.616	Intercept	−18.456	
		Disulfides[a]	0.231	0.0015
	0.901	Intercept	−121.344	
		Disulfides	0.293	0.0001
		Phospholipid[b]	0.376	0.0003
	0.972	Intercept	−107.422	
		Reactive Sulfhydryls	−0.539	0.0001
		Disulfides	0.328	0.0001
		Ash	0.028	0.0001
Sediment fractions	0.965	Intercept	−325.700	
		Calcium[b]	−0.332	0.0054
		Disulfides	0.349	0.0060
		Reactive Sulfhydryls	−1.141	0.0170

[a] In μm/100g final model food formula.
[b] In mg/100g final model food formula.

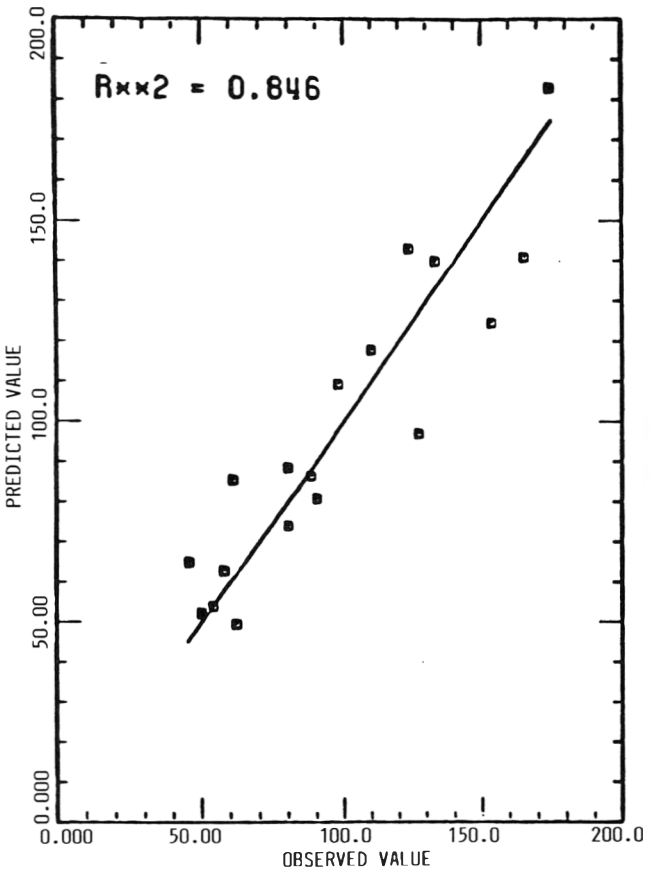

Fig. 1—Goodness of fit for predicting foam capacity (FC) of 1% aqueous solutions of WPC and their soluble and insoluble fractions. Y = 309.48 + 0.135 × X1 − 1.932 × X2 − 0.884 × X3, where Y = foam capacity; X1 = ash, mg/100g dispersion; X2 = phospholipid, mg/100g dispersion; X3 = disulfides, μM/100g dispersion.

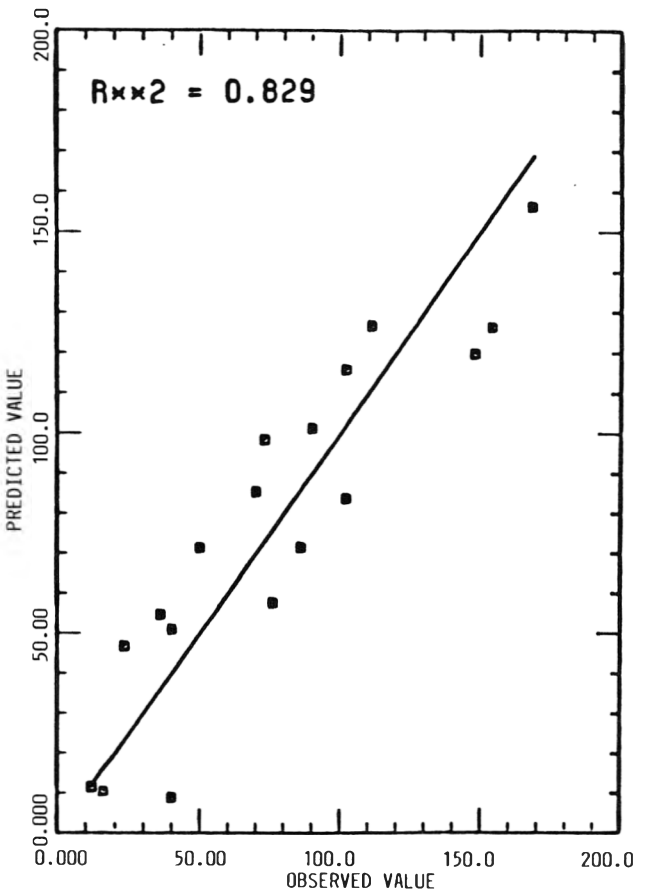

Fig. 2—Goodness of fit for predicting foam stability (FS) of 1% aqueous solutions of WPC and their soluble and insoluble fractions. Y = 282.95 + 0.079 × X1 − 0.366 × X2 − 0.781 × X3, where Y = foam stability; X1 = ash, mg/100g dispersion; X2 = fat, mg/100g dispersion; X3 = disulfides, μM/100g dispersion.

air incorporation when this property of WPC and their soluble and insoluble fractions were measured in distilled water. Foam stability, when determined 15 min after foam formation, was best predicted by the concentration of ash, fat and disulfides. These three variables gave an R^2 value of 0.829 (Fig. 2).

Overrun

When all samples, i.e., WPC and their soluble and insoluble protein fractions were analyzed as one set of data, the best

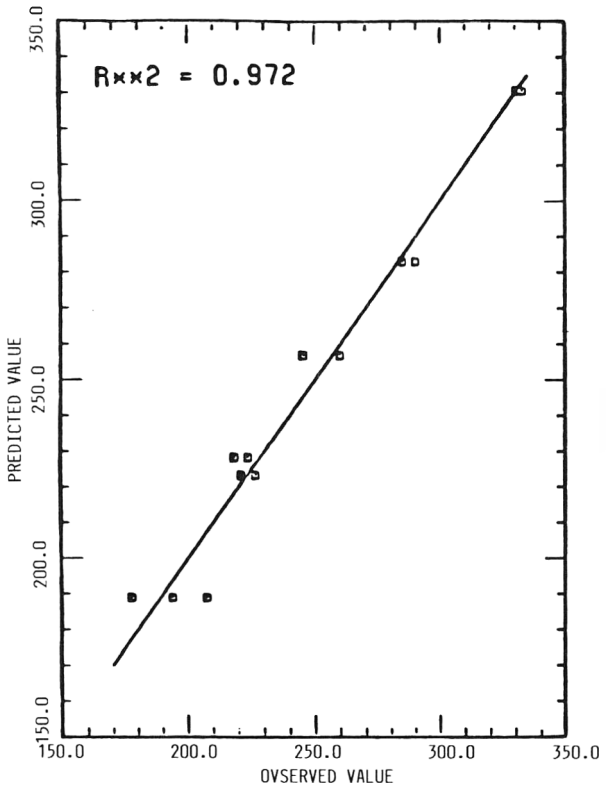

Fig. 3—Goodness of fit for predicting maximum overrun of model food foams stabilized with soluble protein fractions of WPC. Y = − 107.422 − 0.539 × X1 + 0.328 × X2 + 0.028 × X3, where Y = maximum % overrun; X1 = reactive sulfhydryls, μMoles 100g mix; X2 = disulfides, μM/100g mix; X3 = ash, mg/100g mix.

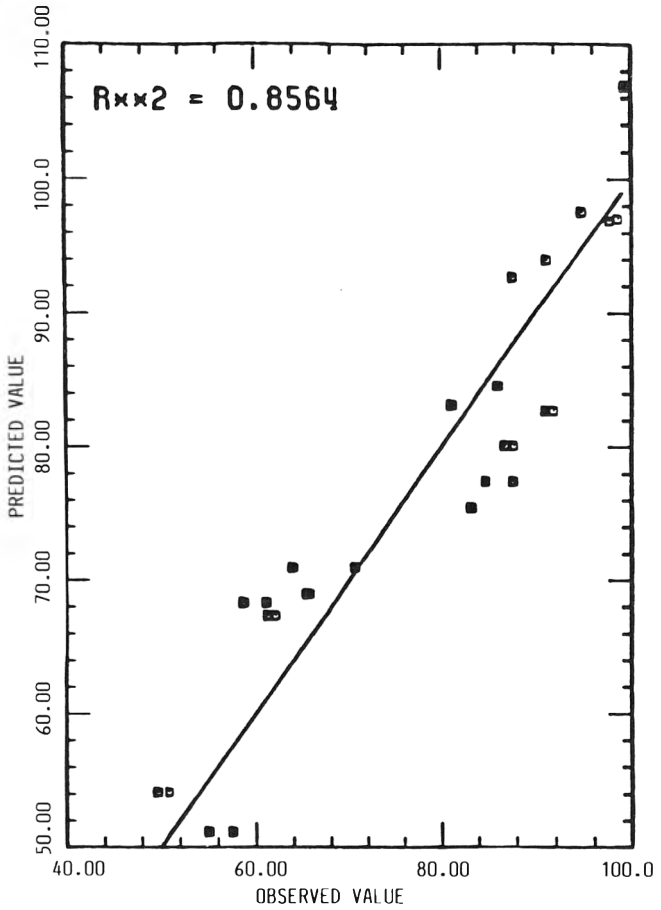

Fig. 4—Goodness of fit for predicting Emulsion Rating of model food emulsions stabilized with WPC and their soluble and insoluble fractions. Y = 97.69 − 4.859 × X1 − 0.006 × X2 + 0.051 × X3, where Y = emulsion rating; X1 = soluble protein, g/100g mix; X2 = ash, mg/100g mix; X3 = calcium, mg/100g mix.

five-variable model for overrun gave a very poor correlation, (R^2 = 0.637). The data were therefore treated as three entities: WPC; the supernatant fraction; and the sediment fraction.

In the case of whole WPC, the first independent variable to be included in the multiple regression equation for overrun was ash (Table 4). With an R^2 value of 0.77, it accounted for 77% of the response. The second variable to be included was free sulfhydryls which increased R^2 to 0.975. The best three-variable model found had ash, fat and reactive sulfhydryls as independent variables. The inclusion of interaction terms or squared terms in the model did not produce further increase in R^2. Similarly, the derived multiple regression equation relating compositional variables to overrun produced by incorporating the supernatant fraction of WPC into the whipped topping, included reactive sulfhydryls, disulfides and ash (Table 4). The goodness of fit for this model is illustrated in Fig. 3.

Generally, when more reactive or free sulfhydryls were available in the protein, the poorer was air incorporation. The disulfide concentration, on the other hand, was positively correlated with overrun. The data obtained by using the sediment fraction of WPC support the above, with disulfide concentration being positively correlated and reactive sulfhydryls negatively correlated with overrun. The value of the model generated by using the insoluble protein fractions as source of protein is questionable, since foaming of these emulsions was very poor. At best this model would indicate which variables govern foaming at a very low overrun range.

Emulsion rating

The fact that functional properties of whey proteins are largely dependent on heat, pH extremes, or mechanical shear introduced during production of concentrates is well recognized. Especially, the emulsion rating value seemed to have been affected by denaturation when the degree of solubility at the average isoelectric point for the mixed protein system was used to provide an index of denaturation. Protein solubility was the most important single variable to explain the performance of WPC and their fractions as stabilizers of emulsions. The higher was the percent of soluble protein, the lower was the emulsion rating after 40 min of centrifugation, with a correlation coefficient of −0.831.

The best three-variable model predicting Emulsion Rating revealed the complicated effect of minerals on properties of whey protein-stabilized emulsions. Ash was negatively correlated with emulsion rating, whereas calcium concentration had a postive correlation with emulsion rating values as shown in Fig. 4. Tornberg (1978) performed a comparison between different proteins as emulsifiers and confirmed that creaming stability of protein-stabilized emulsions showed varying dependence on changes in ionic strength. As a general trend it was found that addition of salt reduced or increased the creaming stability of emulsions, depending on whether they contained WPC or sodium caseinate, respectively. From the data obtained and results published in the literature, it appears that no clear generalizations can be made regarding the effect of minerals on emulsion stability.

Comparison of models predicting foam capacity and overrun

In the simple foaming test, milkfat produced an overall detrimental effect on the foaming properties of WPC and their

fractions. The best three-variable model for foam capacity and foam stability shown in Fig. 1 and 2 illustrates that phospholipids in particular had a negative correlation with foam capacity, whereas total residual fat occurred in the model for foam stability. These results concurred with reports in the literature. There seems to be general agreement that residual lipid inhibits the ability of WPC to function in foaming applications (McDonough et al., 1974; Cooney, 1975; DeWit, 1975; Muller, 1976; Burgess and Kelly, 1979).

When WPC were incorporated into high-fat whipped topping, residual milkfat did not seem to have the role of a foam depressant in this complex system. The best model for overrun of whole WPC included fat as one of the three variables which best predicted overrun, but in this case fat was positively correlated with overrun. Similarly, phospholipid concentration was positively correlated with the good foaming performance of the supernatant fractions. This observation is supported by data of Schmidt and VanHooydonk (1980) who reported the beneficial effects of phospholipids on foaming of cream. If phospholipid concentration was increased, shorter whipping time and a higher overrun were obtained.

Consistently, proteins with greater solubility had higher foam volumes regardless of whether air incorporation was tested in an aqueous dispersion or in the model whipped topping. Also, ash content seemed to be positively correlated with foaming properties of WPC under either simple test conditions or incorporation into the model food.

Tornberg (1978) studied the surface behavior of different protein systems and noted that in the presence of electrolytes, whey proteins diffused faster into the air-water interface than into distilled water. This result could explain the present observation that a higher ash concentration led to a better foam capacity and overrun of whipped topping containing fully or partially soluble WPC. On the other hand, the rapid diffusion of proteins to the interface may have been prevented by high viscosity of samples stabilized with the insoluble fractions of WPC.

Experiments were run to verify the model response. To test the effect of ash on overrun the potassium-neutralized WPC was dialyzed extensively and freeze-dried. Lactose was added back so that ash became the only ingredient in the formulation that was varied. The best model in Table 4 predicted an overrun of 230% before dialysis and one of 180% after dialysis. The observed values were 226% and 190%, respectively, indicating excellent agreement with the model.

The best first-order regression models for the soluble whey protein fractions were developed based on the compositional analysis of the supernatant fractions of the WPC. The laboratory control WPC was not included in developing these models. Using the compositions of the laboratory WPC and the model for the supernatant fractions in Table 5, an overrun of 263 was predicted which was in excellent agreement with the 260% overrun actually obtained. These results indicated that the models generated for the overrun accurately described this high-fat system.

In general, the models generated were consistent with the data of other workers and where tested gave excellent agreement between predicted and observed values. In the case of the high-fat foam system the divisions of the proteins into three fractions caused a significant decrease in the R^2 of the model generated. The fractionation was done to increase the range of compositional factors analyzed in the models. In this case it is apparent that some other factor was also important. It might well be that differences in the protein distribution between the three fractions was important to the functionality of this model system. The case that would probably be of more importance to the manufacturers of WPC would be the examination of a number of closely related whey protein concentrates to determine what characteristics contribute to variability in their functional properties.

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Concentration and Temperature Dependence of Flow Behavior of Xanthan Gum Dispersions

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ABSTRACT

Apparent viscosities of xanthan gum dispersions over shear rates of 0.5–3000s^{-1} were studied at concentrations of 0.05–1.00% (w/w) and temperatures of 5–45°C. All dispersions were shear rate thinning non-Newtonian fluids described accurately by the power-law model. The logarithm of viscosity varied with the logarithm of concentration and the reciprocal of absolute temperature. A combined model was derived to describe the variation of viscosity with shear rate, concentration and temperature for aqueous dispersions of xanthan gum.

INTRODUCTION

THE STRUCTURAL and rheological properties of xanthan gum dispersions have been reported by Rocks (1971), Rao and Kenny (1975), and Tanaka and Fukuda (1976). In recent years many other researchers have also investigated xanthan gum solution properties (Sanford et al., 1977; Whitcomb et al., 1977; Chang and Ollis, 1982; Narayan and Ramasubramanian, 1982; Morris et al., 1983; Southwick et al., 1983; Zatz and Knapp, 1984).

Xanthan gum was developed in the USDA laboratory in Peoria, IL in the early 1960s. The gum is a microbial exopolysaccharide which is produced by the organism *Xanthomonas campestris* in pure culture fermentation. It was first approved for food use by the FDA in 1969. The gum has a number of unique solution properties which make it suitable for use as a food ingredient. The primary function of xanthan gum in most foods is as a viscosity enhancer. When added to fluid foods, it increases low shear viscosity while having little effect on the viscosity of the food at high rates of shear. The phenomenon is known as 'shear rate thinning', or pseudoplasticity which is a desirable property in many fluid foods as it results in superior suspending properties at low shear rates without rendering the food too viscous to mix or pour at higher rates of shear. The gum has found widespread use in a variety of foods such as pie fillings, salad dressings and powdered drink mixes due to its shear thinning properties, tolerance to chemical and processing treatments and effectiveness.

Rocks (1971) reported that xanthan gum was the most pseudoplastic gum available and he and other researchers (Rao and Kenny, 1975; Tanaka and Fukuda, 1976; Sandford et al., 1977; Whitcomb et al., 1977) have used the well known power-law model to characterize the flow behavior of xanthan gum solutions. The power-law model relates apparent viscosity to shear rate and has been widely used to describe flow behavior in many food systems such as tomato products, citrus juices, soups and sauces (Holdsworth, 1971). Apart from the rate of shear, other factors including gum concentration, temperature, salt concentration and gum source can influence xanthan gum solution properties.

Harper and El Sahrigi (1965) investigated the viscous properties of tomato concentrates at a temperature of 32.2°C and a shear rate of 500 s^{-1}. They found that a power-type relationship described the variation in apparent viscosity due to changes in solids concentration between 5.8 and 30% (w/w). Rao and Kenny (1975) have also applied this relationship to the flow behavior of xanthan gum solutions at shear rates of 100 and 500 s^{-1}, and at a temperature of 25°C over a solids concentration of 0.5–1.2% (w/w).

Temperature has an important influence on the flow behavior of xanthan gum solutions. An Arrhenius model was used by Harper and El Sahrigi (1965) to characterize the apparent viscosity of tomato solids at a shear rate of 500 s^{-1}, temperatures of 32.2–82.2°C and solid concentrations of 5.8–30% (w/w). In a review of the literature, Holdsworth (1971) found reports on several fluid foods for which the apparent viscosity change with absolute temperature could be characterized by the Arrhenius model which linearly relates the logarithm of apparent viscosity (at a given rate of shear) to the inverse of the absolute temperature. Rao and Kenny (1975) have applied this model to xanthan gum solutions. They reported that activation energies were both shear rate and concentration dependent. For shear rates of 100 s^{-1} and concentrations of 0.5, 0.75, 1.0, and 1.2% (w/w), they found activation energies of −1.84, −0.95, −0.83, and −1.42 kcal/mole, respectively. Narayan and Ramasubramanian (1982) reported activation energies of 0.5 and 0.9 kcal/mole at a shear rate of 126.9 s^{-1} and concentrations of 1.5 and 2% (w/w). These low activation energies indicate that the apparent viscosity of xanthan gum solutions is not greatly influenced by temperature change.

Harper and El Sahrigi (1965) related the logarithm of apparent viscosity to the logarithm of concentration and inverse of absolute temperature. Chang and Ollis (1982), Narayan and Ramasubramanian (1982) and Zatz and Knapp (1984) have also reported on the effect of limited temperature and concentration changes on xanthan gum solution viscosity.

Other factors which might influence xanthan gum solution properties, such as pH and salt concentration, are reported to have little influence on apparent viscosity in pH ranges of 3.0–11.0 and salt concentrations above 0.1% (Anon., 1975; Zatz and Knapp, 1984). Since shear rate, concentration and temperature are the major factors which influence the apparent viscosity of food dispersions, this study was undertaken to examine the effects of these factors on flow behavior of xanthan gum aqueous solutions. The purpose of this study was to provide a useful data base for solution properties that may be employed as guidelines in formulating foodstuffs containing xanthan gum.

MATERIALS & METHODS

SAMPLES OF XANTHAN GUM (KELTROL, Kelco Division of Merck and Co. Inc., San Diego, CA) were dissolved in water containing 0.15% NaCl and 0.11% $CaCl_2$ to make duplicate solutions of 0.05, 0.10, 0.25, 0.50, and 1.00% (w/w) gum. The solids were dispersed by a high speed blender/sonicator (Polytron, Brinkman Instruments, Rexdale, ON) for 1–2 min. The dispersions were then centrifuged at 10,000 × *g* for 30 min to remove entrapped air bubbles.

Each solution was then subjected to viscometric evaluations using a coaxial cylinder viscometer (Brabender Rheotron, C.W. Brabender Instruments Inc., South Hackensack, NJ). Dupli-

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cate samples were tested at temperatures of 5, 15, 25, 35, and 45°C and shear rates between 0.5 and 3000 s^{-1} using the A1 fixtures with a gap size of 1.0 mm. The samples were loaded, allowed to equilibrate to the water jacket temperature and sheared at a linearly increasing rate from 0 to 3000 s^{-1} in 6.67 min, followed by a linear decrease in shear rate back to zero. During these tests the torsion head and rotational speed signals were recorded with a potentiometric data logger (Digitec Model 1268, United Systems Corp., Dayton, OH) during the 'upcurve and 'downcurve' of each test. Data were obtained from the data logger printout at logarithmic intervals of rotational speed from the upcurve segments to calculate shear rate ($\dot{\gamma}$) and shear stress (σ) values as follows:

$$\dot{\gamma} = 2\Omega \frac{R_c^2}{(R_c^2 - R_b^2)} \quad (1)$$

$$\sigma = \pi \frac{AS}{2\,R_b h} \quad (2)$$

where Ω = angular velocity, R_c = cup radius, R_b = bob radius, A = spring constant, S = scale reading, and h = bob height. The constants were obtained by calibration of the fixture and spring combination with viscosity standards (Cannon Instrument Co., State College, PA).

For each test condition, the flow behavior index (n) was then obtained as the slope of the least squares regression of the logarithm of shear stress on the logarithm of shear rate data. The shear rate calculated using Eq. (1) assumed that the fluid being sheared in the coaxial cylinder gap was Newtonian. These shear rates were then corrected for deviations from Newtonian flow using the Kreiger-Maron method (Van Wazer et al., 1963):

$$\dot{\gamma} = \frac{2\Omega}{n} \frac{R_c^{2/n}}{(R_c^{2/n} - R_b^{2/n})} \quad (3)$$

These corrected shear rates and the shear stress values were then used to first calculate an apparent viscosity (η) which was then used in calculating the flow behavior index and the consistency coefficient (m) by fitting the power-law

$$\eta = m\dot{\gamma}^{(n-1)} \quad (4)$$

to each replicate set of data at each temperature and concentration tested.

Consideration was given to determine whether the gum exhibited an appreciable time dependence. The linear forms of the upcurve and downcurve segments (log η vs log $\dot{\gamma}$) at 1.00% (w/w) and 5°C were subjected to an analysis of covariance procedure (Zar, 1974) to test differences of the slope (n) and intercept (m) values of the two rheograms. Since no significant difference ($p>0.05$) was found, all solutions were assumed not to exhibit time dependence. This covariance procedure was also used to examine duplicate tests for differences in the power-law parameters (m and n). No significant differences ($p>0.05$) existed; thus, the replications were pooled for further data analyses.

Using the power-law parameters obtained, apparent viscosities were calculated at 100 s^{-1}. This shear rate was selected in order to compare data to previous studies (Rao and Kenny, 1975) and because it is a shear rate commonly encountered during stirring and oral evaluations (Sherman, 1975). The derived apparent viscosities were then used to examine viscosity-concentration and viscosity-temperature relationships.

At each of the five temperatures tested the effect of concentration (C) on the apparent viscosity (@ 100 s^{-1}) was examined by fitting the following equation:

$$\eta = aC^b \quad (5)$$

The parameters a and b in Eq. (5) were calculated as the slope and intercept of each of the five regressions of the logarithm of apparent viscosity (@ 100 s^{-1}) vs the logarithm of gum concentration.

The variation in apparent viscosity due to temperature at each gum concentration was also examined using the Arrhenius model:

$$\eta = Ae^{(\Delta E/RT)} \quad (6)$$

where A is the frequency factor, e is the natural logarithm base, ΔE is the activation energy and R is the gas constant. The slopes and intercepts of the regressions of the logarithm of apparent viscosity (@ 100 s^{-1}) vs the inverse of absolute temperature were used to calculate the frequency factors and activation energies at each of the five concentrations examined.

Finally, the combined relationship of apparent viscosity to shear rate, concentration and temperature was examined using multiple linear regression techniques. The logarithm of apparent viscosity was regressed on the logarithm of shear rate, the logarithm of gum concentration, and the inverse of absolute temperature.

RESULTS & DISCUSSION

FOR THE RANGE of shear rates used in this study, the power-law accurately described flow behavior at each experimental concentration and temperature. Consistency coefficients and flow behavior indices for each temperature along with coefficients of determination for each flow curve are shown in Table 1. At each concentration there was a decrease in the consistency coefficient with increasing temperature; however, no trend was evident in the flow behavior index.

Increases in gum concentration increased the consistency coefficient and decreased the flow behavior index, indicating that the solutions became more pseudoplastic as the level of xanthan gum increased. Fig. 1 demonstrates this change in flow behavior with changes in xanthan gum concentration at 25°C.

Higher gum concentrations also resulted in increased apparent viscosity (@ 100 s^{-1}) values at each of the five temperatures tested. When Eq. (5) was applied to the data at each temperature, accurate fits ($p<0.05$) were found for the regression of the logarithm of apparent viscosity on the logarithm of gum concentration. Table 2 contains the parameters a and b for this model as well as the coefficient of determination for each regression.

Table 1—Consistency coefficients and flow behavior indices of xanthan gum dispersions at various temperatures and concentrations

Conc (% w/w)	Temp (°C)	m (mPa·s^n)	n	r^2	No. of data
0.05	5	47.2	0.631	0.979	30
	15	33.2	0.634	0.987	29
	25	25.4	0.654	0.984	28
	35	26.6	0.648	0.981	12
	45	24.0	0.635	0.884	25
0.10	5	228.	0.455	0.933	28
	15	116.	0.507	0.889	27
	25	126.	0.487	0.995	28
	35	101.	0.525	0.989	29
	45	77.4	0.546	0.982	29
0.25	5	1,700.	0.258	0.993	28
	15	1,160.	0.272	0.993	30
	25	971.	0.294	0.994	29
	35	916.	0.293	0.991	30
	45	783.	0.315	0.997	30
0.50	5	2,290.	0.250	0.988	26
	15	2,090.	0.252	0.992	27
	25	2,160.	0.249	0.994	28
	35	2,210.	0.234	0.993	28
	45	1,980.	0.249	0.997	30
1.00	5	5,750.	0.232	0.993	27
	15	7,930.	0.178	0.994	29
	25	10,100.	0.141	0.995	30
	35	9,540.	0.149	0.996	30
	45	8,220.	0.164	0.998	30

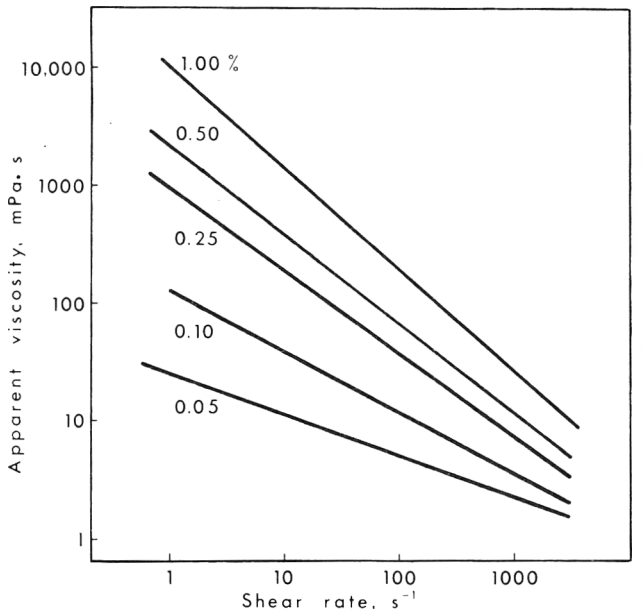

Fig. 1—Effect of shear rate on apparent viscosity (@ 100 s⁻¹) at 25°C for 0.05–1.00% (w/w) xanthan gum dispersions.

Table 2—Apparent viscosity (@ 100 s⁻¹) as a function of concentration for xanthan gum dispersions from 5 to 45°C

Temperature (°C)	a (mPa·s%$^{-b}$)	b	r^2
5	167.	0.963	0.982
15	169.	1.12	0.993
25	181.	1.18	0.996
35	163.	1.09	0.973
45	163.	1.20	0.994

Table 3—Apparent viscosity (@ 100 s⁻¹) as a function of temperature for xanthan gum dispersions at concentrations of 0.05 to 1.00% w/w

Conc (% w/w)	ΔE (kcal/mole)	A (mPa·s)	r^2
0.05	1.87	0.263	0.881
0.10	2.40	0.211	0.791
0.25	2.04	1.28	0.851
0.50	0.523	27.6	0.530
1.00	−0.264	283.	0.116

When the apparent viscosity values (@ 100 s^{-1}) were examined with respect to temperature, it was found that the apparent viscosity decreased with increasing temperature. When the Arrhenius model (Eq. 6) was fitted to the data, significant relationships ($p<0.05$) were found at all but the 1.00% concentration level. The activation energies, frequency factors and coefficients of determination are shown in Table 3. Fig. 2 contains these rheograms for the five gum concentrations studied along with the line for pure water for comparative purposes. The interesting feature of this plot is that xanthan gum solutions had only small viscosity changes with increasing temperature and that the temperature effect decreased with increasing gum concentration.

The activation energies reported in this study differ from the values of Rao and Kenny (1975) and Narayan and Ramasubramanian (1982). These discrepancies may be due to differences in xanthan gum type, dispersion preparation methods, shear rate, and temperature ranges examined.

The relationships of apparent viscosity to shear rate, concentration and temperature were combined in an equation similar to that used by Harper and El Sahrigi (1965). Using multiple regression, the relationship obtained was:

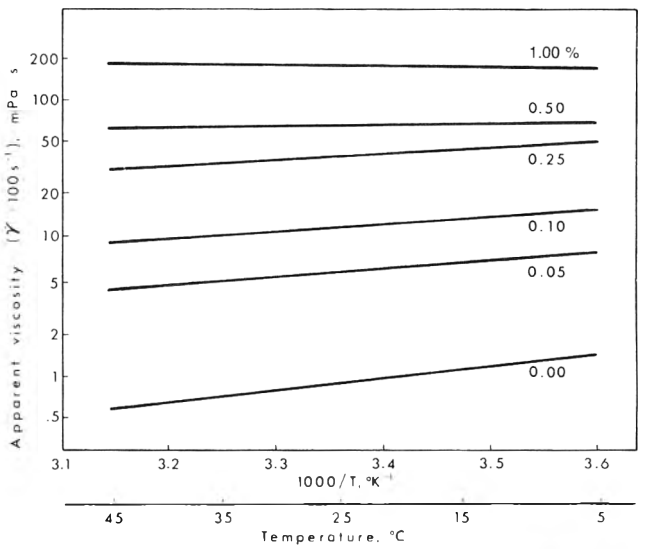

Fig. 2—Effect of temperature on apparent viscosity (@ 100 s⁻¹) for water and 0.05–1.00% (w/w) xanthan gum dispersions.

$$\eta = 396 \cdot \gamma^{0.642} C^{1.22} e^{668/T} \quad (7)$$

The units for apparent viscosity are mPa s, C is % w/w and T is in °K. This model fitted all the data accurately as indicated by a coefficient of multiple determination of 0.946 for a total of 697 data points.

It is hoped that the derived models can be used in food product research and development. When formulating a food dispersion to provide desired textural attributes, a trial and error approach is often used. In many cases the approximate shear rates and end-use temperature of a product will be known. With Eq. (7), it would be possible to find the viscosities obtained with varying concentrations of gum, and thereby select a concentration that will provide a suitable viscosity. Trials with other ingredients will be required to settle upon an approximate formulation; however, this approach may provide a first estimate of the amount of gum needed and should reduce the time spent in achieving the desired textural properties for a product.

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Effect of Deamidation and Succinylation on Some Physicochemical and Baking Properties of Gluten

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ABSTRACT

Vital wheat gluten was modified by deamidation and succinylation. Deamidation caused a progressive degradation of gliadin with concomitant increase in low molecular weight components, but glutenin was not affected. Deamidation also markedly increased the net negative charge and surface hydrophobicity of gluten, while the bread loaf volume and dough extensibility were decreased. The most significant change in physiochemical properties of gluten caused by succinylation was an increase in net negative charge. Succinylation led to a pronounced decrease in dough extensibility but no significant changes in specific loaf volume. The data indicated the importance of hydrogen bonding offered by the amide groups of gluten in the breadmaking process. Changes in molecular weight distribution and hydrophobic interaction may also affect the baking performance of gluten. Ionic interaction may be involved in dough development but is less critical in controlling the overall baking performance of gluten.

INTRODUCTION

IT IS WELL RECOGNIZED that wheat gluten plays a principal role in breadmaking. However, the reasons why gluten from different wheat varieties has diverse baking quality are not clearly understood. The precise ways in which gluten components interact during the baking process are also far from resolved. A number of covalent (disulfide) and noncovalent (hydrogen, ionic, hydrophobic) interactions have been implicated as essential in dough mixing and bread baking (Ewart, 1968; Mecham, 1980; MacRitchie, 1979; Kobrehel and Bushuk, 1977). Physical and chemical properties of gluten such as molecular size, gliadin/glutenin ratio and degree of primary amidation have also been suggested as factors affecting baking quality (Cunningham et al., 1955; Holme and Briggs, 1959; Payne et al., 1979; Orth and Bushuk, 1972; MacRitchie, 1979; Huebner and Wall, 1976).

One approach to such studies is to change the physicochemical properties of gluten by chemical modification. Wheat gluten has been modified by deamidation (mild acid or alkaline hydrolysis) and succinylation, leading to changes in functional properties and baking quality (Gagen and Holme, 1973; Wu et al., 1976; Batey, 1980; Matsudomi et al., 1981; Barber and Warthesen, 1982; Gebhardt et al., 1982). However, not much information is available on the physicochemical characteristics of these modified proteins. The present study was undertaken to investigate the effect of deamidation and succinylation on some physicochemical properties of gluten, including amide content, net charge, molecular weight distribution and surface hydrophobicity. These changes were correlated to the dough mixing and baking performance of gluten in an attempt to evaluate the importance of certain intermolecular bondings in the breadmaking process.

MATERIALS & METHODS

Preparation of deamidated gluten

Vital wheat gluten (Industrial Grain Products, Montreal, Canada) was hydrolyzed by heating under reflux for 2 hr a 5% (w/v) gluten suspension in HCl with strength varying from 0.02N to 1N. The hyrolyzed gluten solution was neutralized with 2N NaOH to pH 7, dialyzed exhaustively against distilled water at 4°C and freeze-dried. Under such mild acid treatments, loss of amide groups from glutamine and asparagine was observed without extensive peptide bond cleveage (Gagen and Holme, 1973; Wu et al., 1976).

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Preparation of succinylated gluten

A 5% (w/v) aqueous gluten suspension was adjusted to pH 8 with 2N NaOH, and solid succinic anhydride was added while stirring and maintaining pH at 8.0 with 2N NaOH until all anhydride has reacted as indicated by no more change in pH. The amount of anhydride added was 1g, 4g, 8g, and 20g/100g gluten. The reaction mixture was dialyzed with frequent changes of distilled water at 4°C for 48 hr and then freeze-dried.

Chemical analyses

Amide nitrogen was determined as distillable ammonia after refluxing the gluten in 2N HCl for 3 hr (Wilcox, 1967). Kjeldahl nitrogen was determined by the method of Concon and Soltess (1973). The extent of succinylation was determined by measuring the free available lysine in the protein samples, using the dinitrobenzenesulfonate (DNBS) method (Concon, 1975).

Column chromatography

Modified gluten samples were fractionated on Sephacryl S-200 (Pharmacia Fine Chemicals, Uppsala, Sweden) column (2.5 × 45 cm), equilibrated and eluted with 2M sodium thiocyanate as described by Preston (1982). The column was calibrated with standard proteins of known molecular weights, including human γ-globulin, bovine serum albumin, ovalbumin, trypsin inhibitor, and cytochrome C.

pH-Titration

pH-Titration of native and modified gluten was carried out on 1.0% protein solutions in 0.1M NaCl. The pH of the protein solution was adjusted to 10.0 and 0.1N HCl was added until an arbitrary end point of pH 3.0 was reached.

Measurement of surface hydrophobicity

Modified gluten solutions (2 mg/10 mL) were prepared in 0.01M phosphate buffer, pH 7.4 by homogenizing at low speed in a Sorvall homogenizer. The solutions were centrifuged at 5,000 X *g* and the protein content in the supernatant was determined by a micro-Kieldahl procedure (Concon and Soltess, 1973). Approximately 80–90% of gluten proteins were soluble in the buffer. The protein solutions were used for determination of the surface hydrophobicity using the fluorescence probe method of Kato and Nakai (1980).

Bread baking tests

In order to assess the effect of modified glutens in a bread baking system, a soft wheat flour was used as a base, to which glutens were subsequently added. A commercial soft wheat flour (Everlite, Reid Milling, Mississauga, Ont.) with protein of 8.5% (N X 5.7) and ash of 0.48% on a 14% moisture basis (mb) was used for this purpose.

Breads were baked by a straight-dough procedure (Oomah, 1983) using the following formulation: wheat flour (14% mb), 100g; compressed yeast (Lallemand, Canada), 3.5g; sugar, 3.3g; shortening (Crisco, Procter and Gamble, Canada), 3g; salt, 1.7g; Delquik 70 HS (a dough conditioner containing 68 ppm of ascorbic acid, 40 ppm of potassium bromate, and 12.5 ppm of cysteine-HCl, Ogilvie Flour Mills Ltd., Canada), 0.5g; malt, 0.3g; ammonium phosphate mono-

basic, 0.1g; and water calculated to 52.5% absorption. Vital or modified gluten was added at levels of 1, 2, 3, and 4% (flour weight basis) to the formula. Doughs were mixed for 3.5 min in a National 100–200g mixer (National Mfg. Co., Lincoln, NB). Rest time was 60 min at 28°C and 85% relative humidity (R.H.). The fermented doughs were sheeted with a National sheeter, molded in a SH-E-L molder (National Mfg. Co., Lincoln, NB) and panned. After a 90-min proof time at 28°C, the bread was baked for 25–30 min at 218°C in a reel rotating oven (National Mfg. Co., Lincoln, NB). Volumes of the cooled loaves were measured by rapeseed displacement.

Extensograph properties

Extensograms were obtained according to AACC method 46.10 (1961) with some modifications. Wheat gluten (vital and modified) was reconstituted with wheat starch (Whetstar 4, Ogilvie Mills Ltd., Montreal, Canada) to obtain a protein content of 12% expressed on a 14% moisture basis. Doughs were made from 300g of reconstituted flour (14% mb) and distilled water equal to farinograph absorption. Measurements were made for duplicate doughs after a rest period of 60 min. Flour containing deamidated gluten, particularly the highly modified samples, formed very weak dough, and extensograph measurements could not be made.

RESULTS

Extent of modification

Table 1 shows the yield and extent of deamidation of gluten treated with acid. The yield represents the amount of protein recovered which decreased gradually with increase in HCl concentration. This was related to the extent of hydrolysis of the protein and the loss of amino acids and low molecular weight peptides through dialysis.

Figure 1 shows the extent of succinylation of gluten estimated as the amount of free lysine acylated. The highest level of modification, with the addition of 20g anhydride/100g gluten, was approximately 60%. A previous report (Barber and Warthesen, 1982) showed that even at an anhydride concentration at 1g/2g gluten, only 60% of the free amino groups in gluten can be acylated.

Table 1—Yield and extent of deamidation of acid hydrolyzed gluten[a]

HCl Conc	Yield of N (%)	Extent of deamidation (%)
0.02N	92.0	9.8
0.05N	91.4	28.8
0.15N	88.4	58.1
0.30N	73.1	81.4
1.00N	43.3	91.5

[a] Average of duplicate determinations.

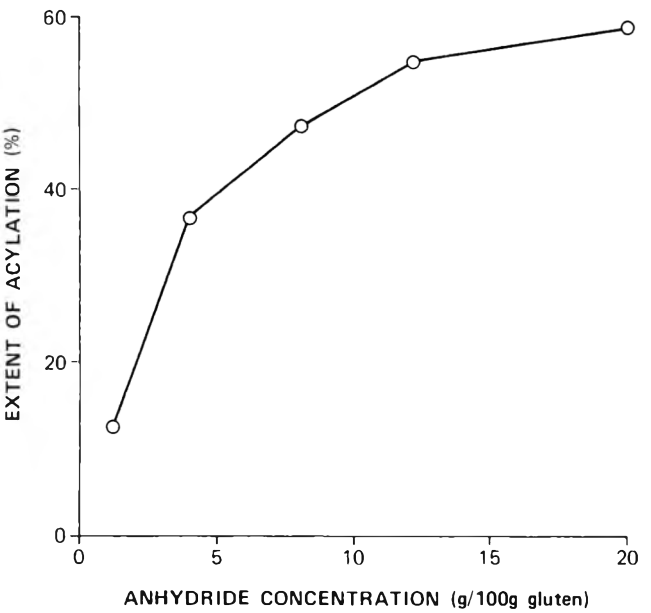

Fig. 1—Effect of succinic anhydride concentration on the extent of acylation of gluten.

Molecular weight distribution of modified gluten

Figure 2 shows the gel filtration chromatographic patterns of unmodified and deamidated gluten samples fractionated on a Sephacryl S-200 column, using 2M sodium thiocyanate as eluting buffer. The pattern of the unmodified gluten was similar to that reported by Preston (1982) using a Sephacryl S-300 column. There were two large peaks with high molecular weight (>60,000) and two smaller peaks with lower molecular weight (<30,000), and some minor fractions with molecular weight less than 10,000. Succinylation, even at the highest level, did not alter the elution profile of gluten (pattern not shown). Deamidation caused a gradual decrease in the size of the second peak which contained mainly gliadin-like proteins (Preston, 1982) and a concomitant increase in the smaller molecular weight fractions. However, there was no significant change in the first peak which contained essentially glutenin (Preston, 1982). The results indicated that succinylation did not cause marked dissociation of the protein molecules, whereas gliadin was more susceptible to acid hydrolysis than glutenin. Using reconstitution techniques, Batey (1980) also demonstrated that the gliadin fraction is particularly susceptible to alkaline hydrolysis (0.2M NaOH, 2 hr).

pH-Titration

Table 2 shows that net titratable charge of native and modified vital gluten by titration of the samples from pH 10.0 to 3.0. The result showed that deamidation and succinylation caused a progressive increase in the bound H^+ with an increase in the level of modification. Both the removal of amide groups by acid hydrolysis and the modification of lysine ϵ-amino groups (and to a lesser extent the SH groups of cysteine and phenolic OH groups of tyrosine) by succinylation led to an increase in free carboxyl groups and net negative charge. However, the result indicated that deamidation caused much more pronounced increases in the net charge than succinylation.

Surface hydrophobicity

The changes in surface hydrophobicity of gluten modified by acid hydrolysis and succinylation are shown in Fig. 3. Both

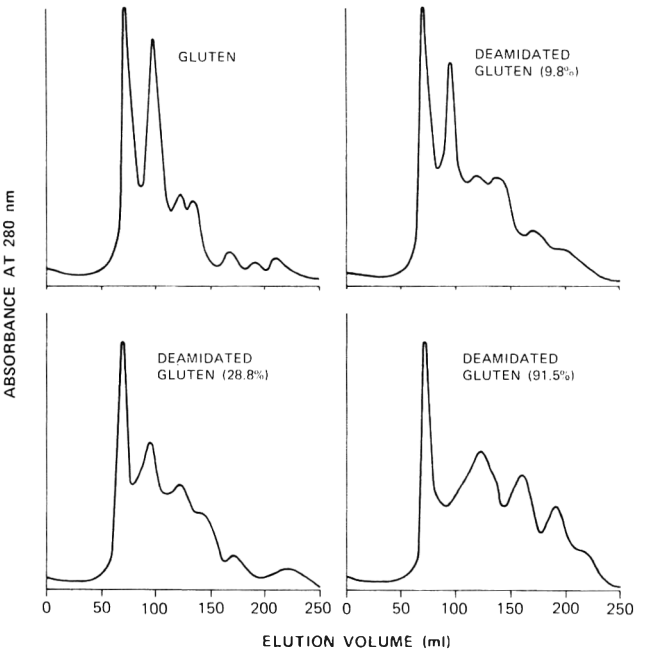

Fig. 2—Molecular weight distribution of deamidated gluten. The proteins were fractionated on 2.5 X 45 cm Sephacryl S-200 column with 2M sodium thiocyanate. Flow rate was 40 mL/h.r.

Table 2—Net titratable charge of native and modified vital gluten[a]

Sample	Net titratable charge (m moles H^+/g protein)
Vital wheat gluten	0.24
Deamidated gluten, 9.8%	0.34
Deamidated gluten, 28.8%	0.61
Deamidated gluten, 58.1%	1.15
Deamidated gluten, 81.4%	1.47
Succinylated gluten, 13.7%	0.25
Succinylated gluten, 37.6%	0.35
Succinylated gluten, 48.5%	0.42
Succinylated gluten, 57.1%	0.48

[a] pH-Titration was performed from pH 10.0 to 3.0. Values are average of two determinations.

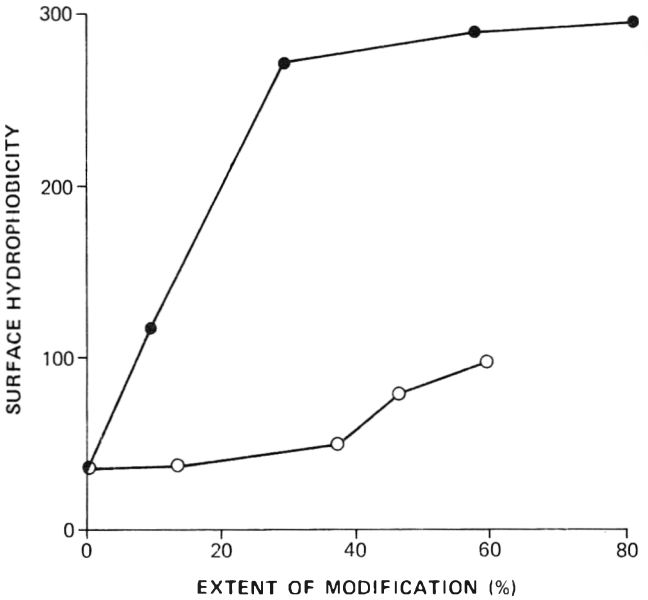

Fig. 3—Effect of deamidation and succinylation on the surface hydrophobicity of gluten: ●; deamidated; ○; succinylated.

deamidation and succinylation may cause conformational changes in gluten proteins and exposure of buried hydrophobic groups and hence yield an increase in surface hydrophobicity. The results showed that deamidation caused a more dramatic increase in surface hydrophobicity than succinylation. Matsudomi et al. (1982) found a linear increase in surface hydrophobicity with increase in the degree of deamidation up to 40%. The present data showed that above 30% deamidation, there was much slower increase in the surface hydrophobicity. Extensive hydrolysis of gluten may lead to the exposure of a large number of hydrophilic groups which could affect the binding of the fluorescence probe to the exposed hydrophobic groups resulting in an apparently lower hydrophobicity reading. The surface hydrophobicity of the succinylated gluten did not increase significantly except at the higher level of modification. This again suggested that succinylation of gluten did not cause extensive dissociation or unfolding of the protein molecules.

Bread baking tests

Figures 4 and 5 show the effect of deamidation and succinylation, respectively, on specific loaf volume (SLV). A marked decrease in SLV was observed even with 9.8% deamidation. The lowest SLV was observed with 28.8% deamidation, and there was a slight recovery at 58.1% modification (Fig. 4). Highly deamidated (80–90%) gluten did not produce a workable dough, and no baking data were presented. When subjected to a best-fit curve analysis, the data followed a polynomial equation with a highly significant correlation coefficient

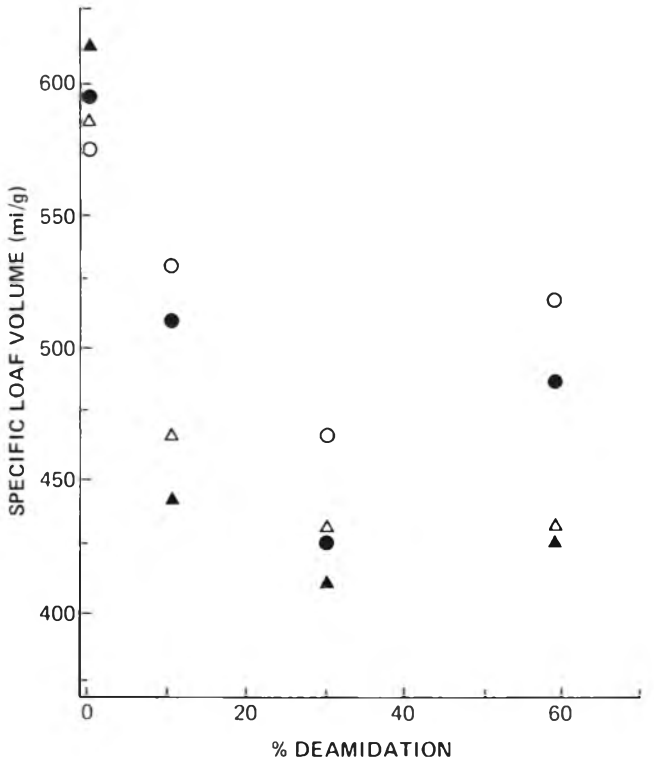

Fig. 4—Effect of deamidation of gluten on the specific loaf volume. Level of gluten addition: ○, 1%; ●, 2%; △, 3%; ▲, 4%. The best-fit equation was $y = 588.8 - 7.628x + 0.094 X^2$; $r = 0.998$.

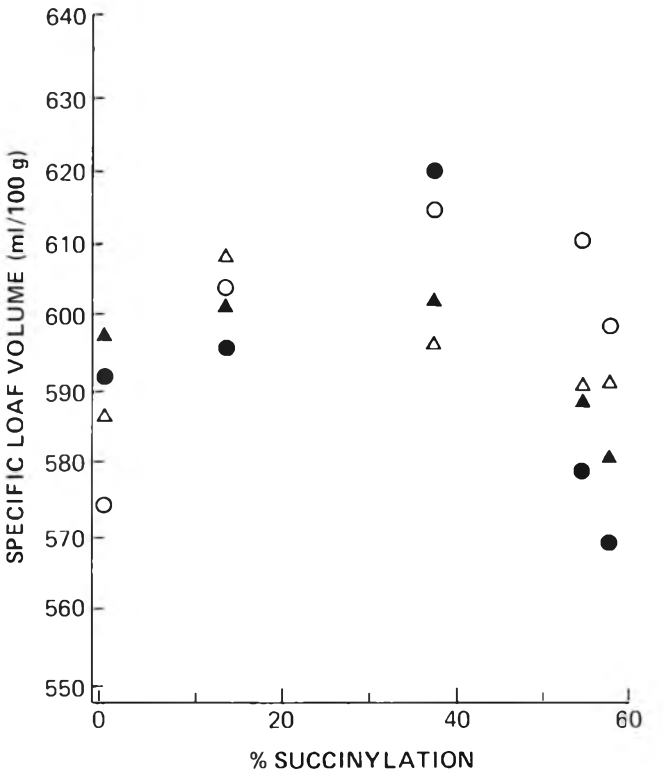

Fig. 5—Effect of succinylation of gluten on the specific loaf volume. Level of gluten addition: ○, 1%; ●, 2%; △, 3%; ▲, 4%. The least square regression equation was $y = 596.9 - 0.393x$; $r = 0.316$.

($r = 0.998$, $P \leq 0.01$). The effect of adding different levels (1–4%) of gluten on SLV was found to be insignificant.

Succinylation caused a slight increase in SLV at lower level of modification and then a decrease to values close to those of the control at higher level of acylation (Fig. 5). No significant correlation ($r = 0.316$) could be found between SLV and degree of succinylation.

Extensograph properties

Table 3 shows the effect of succinylation on extensograph properties of gluten. Extensibility (E) decreased with increase in the level of succinylation. The resistance (R) was not changed by mild succinylation (13.7%) but was markedly decreased at higher level of succinylation (37.6%). The ratios of extensibility to resistance (R/E) of the two succinylated gluten samples were about the same and were approximately twice that of the vital gluten. The weak dough property of the succinylated gluten samples was also evidenced by the small extensograph area when compared to the unmodified control.

DISCUSSION

THE PRESENT DATA showed that deamidation and succinylation caused changes in the physiochemical properties of vital gluten, including net charge and surface hydrophobicity and for deamidated gluten, molecular weight distribution. All the changes were more pronounced with deamidation. Succinylation has been shown to cause extensive changes in conformation, physicochemical and functional properties of some plant proteins including peanut, cottonseed, soybean, wheat and oat (Kinsella and Shetty, 1979). Gluten has a low lysine content (2%), and the extent of succinylation was less than 60%. Compared to deamidation, succinylation did not cause as extensive an increase in net charge and related physicochemical changes.

The present data showed that deamidation of gluten caused significant decrease in loaf volume and complete loss of extensibility of the dough. This is consistent with previous reports (Gagen and Holme, 1973; Batey, 1980; Gebhardt et al., 1982). Deamidation of vital gluten using HCl at 0.15N, 0.30N and 1.00N under reflux for 2 hr reduced the relative baking performance (compared to that of vital gluten) to 0.75, 0.61 and 0.43 respectively (Gagen and Holme, 1973). Gebhardt et al. (1982) reported that addition of 3% deamidated gluten decreased elasticity and extension resistance of the dough and reduced loaf volume. Batey (1980) showed a loss of mixing and baking properties in deamidated, reconstituted gluten. The baking data of the succinylated gluten are also consistent with those of Gagen and Holme (1973) who reported that the relative baking performance of succinylated gluten (modified at one level) was 1.02 compared to that of vital gluten (added at the 4% level to the baking formula). By modifying gluten to various degrees and using four levels of gluten substitution, our data present a more detail assessment of the effect of deamidation and succinylation on the baking performance of wheat gluten.

Due to the large number of amide groups in gluten, hydrogen bonding had been implicated as the major secondary force involved in the interactions among wheat flour proteins and between proteins and other flour ingredients (Cunningham et al., 1955; Holme and Briggs, 1959). However, there are conflicting reports on the relationship between baking quality and the amide content in different wheat varieties (MacRitchie, 1979; Ewart, 1982). A highly significant ($r = 0.756$, $p \leq 0.01$) correlation was found between gluten amide content and loaf volume index for 37 samples of Australian flours covering a wide range of baking quality (MacRitchie, 1979). However, a later study on 36 English-grown wheat samples showed no correlation between gliadin/glutenin ratios (which are positively related to gluten amide level) and loaf volume. The major difficulty in these types of studies was a low variability (less than 3%) in the amide level in various wheat varieties. In the present study, the amide contents in the unmodified and deamidated gluten samples covered a much wider range, and the effect on baking performance can hence be more accurately assessed. The present data confirmed the importance of amide groups and hydrogen bonding forces in the breadmaking process.

Lasztity (1969) showed an increase in viscosity of gluten solutions in 1.0N acetic acid with increase of deamidation from 50% to 100%. Our baking data showed a minimum loaf volume at 28.8% deamidation followed by a slight increase at 58.1% modification. This recovery in loaf volume could be due to the decrease in dough viscosity of the highly deamidated gluten.

The marked changes in molecular weight distribution of deamidated gluten could affect the baking quality through an influence on functionality such as solubility. The ability to form high molecular weight aggregates has been regarded as an important property of gluten in breadmaking (Kasarda et al., 1976), and this could also be affected by extensive decrease in the molecular size of the gluten polypeptides. The fact that gliadin was selectively hydrolyzed suggested that baking performance can be lowered without degradation of glutenin. Previous reports (Holme and Briggs, 1959; Batey, 1980) showed that gliadin was preferentially hydrolyzed, and deamidation of gliadin alone could decrease the breadmaking performance of gluten.

Both deamidation and succinylation caused a marked change in the net charge of gluten which may affect the breadmaking quality. Batey (1983) reported that modification of gluten with potassium cyanide caused a reduction in mixing and baking performance and attributed the changes to the shift in net charge due to the elimination of the positive ϵ-amino groups of lysine. A lowering of pH has also been found to decrease the extensibility of doughs (Bennett and Ewart, 1962; Tsen, 1966), suggesting that the increase in electrostatic repulsive forces may decrease interaction between protein molecules. The present data showed a pronounced reduction in dough extensibility by succinylation and its complete loss in the deamidated gluten. While deamidation caused changes in other physicochemical properties, e.g., decrease in primary amide content and decrease in the molecular size of gluten polypeptides, which may have an influence on the mixing process, the only major change in the succinylated gluten was an increase in net charge. Hence, the data strongly suggested that electrostatic interaction was important in controlling dough development. Although deamidation caused more pronounced increases in net charge than succinylation (Table 2), the moderately and highly succinylated gluten had net charge values higher than that of the mildly deamidated (9.8%) sample. However, the specific loaf volume was significantly decreased only by deamidation, indicating that ionic interaction may not be a major factor in controlling the overall baking performance of gluten.

The increase in surface hydrophobicity by deamidation seemed

Table 3—Effect of succinylation on extensograph properties of gluten

Sample	Resistance, R (BU)[a]	Extensibility, E (cm)	Resistance to extension, R/E	Area (cm^2)
Vital wheat gluten	548	92	5.9	64.2
Succinylated gluten 13.7%	558	50	11.2	33.7
Succinylated gluten 37.6%	430	38	12.0	19.1

[a] Brabender units

to coincide with the decrease in SLV, both with an initial dramatic change at lower degree of modification followed by a levelling off. An increase in hydrophobicity in highly succinylated gluten also corresponded to a slight lowering in SLV. Increases in the surface hydrophobicity of gluten may interfere with the intermolecular interactions between gliadins and glutenins which are vital in the baking process. Hydrophobic interaction has been suggested as an important secondary force in breadmaking (Khan and Bushuk, 1979; Wall, 1979), and the present data provided some evidence in support of this hypothesis.

The functional properties of gluten have been found to be markedly changed by deamidation and succinylation (Gagen and Holme, 1973; Wu et al., 1976; Matsudomi et al., 1981; Barber and Warthesen, 1982). Some of these changes, e.g., solubility and water absorption, may have profound influence on gluten behavior in the dough. The fact that the baking performance was not markedly altered by succinylation suggested that wheat gluten has tolerance towards changes in some functional properties, if level of primary amide groups is retained near normal.

CONCLUSION

BY MODIFYING the physicochemical characteristics of gluten through deamidation and succinylation, gluten products with a wide spectrum of amide content, net charge, molecular weight distribution and surface hydrophobicity can be produced. Modifications that caused a loss of primary amide groups and lowering of molecular size led to detrimental changes in dough development and baking properties. Modifications which increased net negative ionizable groups but did not remove primary amide groups led to loss of dough development properties but did not significantly affect the baking properties of gluten.

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Effect of Heating Rate on Thermally Formed Myosin, Fibrinogen and Albumin Gels

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ABSTRACT

Myosin, fibrinogen and albumin gels were formed by heating in pH 6.0 phosphate buffer at three heating rates. Turbidity (A_{660nm}) and solubility were monitored along with gel strength, as measured with an annular pump. Myosin and fibrinogen suspensions became turbid and solubility decreased as temperatures preceding the development of gel strength. Linearly increasing heating rates of 12°C/hr and 50°C/hr produced the strongest myosin and fibrinogen gels at 70°C, whereas albumin gels formed at 95°C by heating at 12°C/hr or constant heating for 20 min did not differ in strength.

INTRODUCTION

BLOOD COLLECTED from meat animals at slaughter has received considerable attention concerning its use as a food ingredient (Satterlee, 1981). Various collection and fractionation techniques have been used to produce both plasma- and red blood cell-based protein preparations (Tybor et al., 1973, 1975; Donnelly and Delaney, 1977; Graham, 1978; Imeson et al., 1978; Danish Meat Research Institute 1980, 1981; Etheridge et al., 1981). Plasma functionality has been studied in emulsions (Satterlee and Free, 1973; Tybor et al., 1973, 1975; Comer, 1979; Terrell et al., 1979; Caldironi and Ockerman, 1982), foams (Tybor et al., 1975; Etheridge et al., 1981) and gels (Harper et al., 1978; Comer, 1979; Hickson et al., 1980, 1982). Plasma has also been used as a meat binding agent (Suter et al., 1976; Seideman et al., 1979; Siegel et al., 1979; Howell and Lawrie, 1984). Plasma contains a heterogeneous mixture of proteins with different chemical and physical properties. Penteado et al. (1979) showed that food functionality varied among plasma protein fractions, with albumin having better foaming properties and globulin being a better emulsifier.

Myofibrillar proteins play a major functional role in production of processed meats and myosin is generally considered as the most effective protein (Fukazawa et al., 1961; Sato and Nakayama, 1970; Nakayama and Sato, 1971a, b; Schmidt et al., 1981; Acton et al., 1983). Binding junctions that would develop between meat pieces in sectioned and formed products were shown to be strongest when formed with myosin (Macfarlane et al., 1977; Ford et al., 1978; Siegel and Schmidt, 1979a, b; Turner et al., 1979). Strength of protein gels is a criterion that is frequently used to evaluate food proteins. Consequently, extensive work has been done to quantify the effect of pH, temperature, ionic strength and protein concentration on myosin gelation (Samejima et al., 1969, 1981; Tsai et al., 1972; Yasui et al., 1979; Ishioroshi et al., 1979, 1981).

Longtime–low temperature cookery is widely used in the meat industry to improve tenderness (Laakkonen, 1973) and to improve product yields through decreased moisture losses (Bayne et al., 1973; Appel and Lofqvist, 1978). However, in some studies slower heating rates have been shown to increase moisture loss (Bramblett and Vail, 1964; Locker and Daines, 1974); thus, other variables such as size of cut, method of heating, meat pH and freezing may be important in determining moisture losses at different heating rates.

There were two objectives in this study. In the first objective, the plasma proteins, albumin and fibrinogen, were evaluated for their ability to form thermally induced protein gels in a buffer system that simulated the aqueous environment of processed meat. These gels were compared to myosin gels. The second objective was concerned with the effect of heating rate on the formation of myosin, albumin and fibrinogen gels.

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MATERIALS & METHODS

Buffer system

All proteins were suspended in a buffer designed to simulate the aqueous phase of processed meat. The buffer consisted of 50 mM sodium phosphate buffer, pH 6.0, 0.5M NaCl and 0.02% NaN_3.

Protein preparations

Unless specifically indicated, all protein preparations were done at 4°C. Porcine myosin from muscles of the rear leg was prepared by using a modification of the method of Quass and Briskey (1968). In the modified method, three precipitations at 0.23M KCl were used to separate actomyosin from myosin. The myosin was used within four days of isolation.

Blood was collected at a local slaughter plant using a citrate anticoagulant (sodium citrate, 89.6 mM; dextrose, 141 mM; citric acid, 15.6 mM; monobasic sodium phosphate, 16.6 mM) in a ratio of 140 mL anticoagulant to 1L of blood. Bovine plasma was separated by two centrifugations at 5,000 × *g* for 20 min. Fibrinogen was isolated from plasma according to the method of Silberstein et al. (1973), with centrifugation rather than filtration being used for clarification. Bovine serum albumin (fatty acid free) was obtained from Sigma Chemical Company, St. Louis, MO. All proteins were placed in buffer and dialyzed overnight against the same buffer. Prior to heating, protein suspensions were clarified (17,000 × *g* for 1 hr) and then adjusted to 3 or 6 mg/mL. Protein preparations were stored at 4°C and checked for purity with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Heating runs

The protein suspensions were distributed in 5 mL quantities in 16 × 100 mm disposable culture tubes. Plastic caps were placed on the tubes to prevent evaporation during heating. For the linearly increasing heating experiments of 12°C/hr and 50°C/hr, the tubes were placed in a water bath at 20°C and allowed to equilibrate for 5 min prior to starting the heating. A recording thermometer was used to monitor the temperature of buffer (5 mL) in culture tubes (16 × 100 mm) and water bath temperature. Thermocouples were placed in the geometric center of the buffer. Four thermocouple-containing culture tubes and two thermocouples to monitor water temperature were randomly distributed in the water bath for each heating run. Linear heating runs were produced by drawing a straight line on temperature recording chart paper between the initial (20°C) and final temperature. The distance between the initial and final temperatures corresponded to the time required to produce a 12°C/hr or 50°C/hr run, taking into account the chart speed. The water bath regulator was continuously adjusted so that both water and buffer temperatures followed the line. These rates were always linear and henceforth will be referred to as 12°C/hr and 50°C/hr. In the constant temperature heating experiments, tubes

were removed from a 4°C cold room, placed in a water bath at the desired temperature and held for 20 min. The temperature of buffer within tubes was 99% of bath temperature after 2 min. Linear heating samples were removed from the water bath when the buffer reached the desired temperature. After heating, the tubes were placed at 4°C and held overnight. Samples were run in duplicate and each treatment had a minimum of two replications, most had three.

Gel strength

An annular pump connected to an Instron (Model 1122) Universal Testing device, described by Harper et al. (1978), was used to determine gel strength. Gel strength was determined by plunging a 9.5 mm diameter stainless steel rod at a constant rate of 100 mm/min into the protein-gel contained in a 16 × 100 mm tube to a distance 3 cm below the gel surface. Gels were held at 4°C for gel strength measurement. The area under the force-penetration curve for a 3 cm penetration was found to be consistent between duplicate and replicate samples; hence gel strength is reported as work (joules) for a 3 cm plunge. The area under the curve was determined with a Hewlett-Packard digitizer (model 9107A) coupled to a Hewlett-Packard calculator (model 9100B).

Turbidity

Turbidity ($A_{660\ nm}$) was determined on ruptured gels/protein suspensions at room temperature after gel strength testing. Samples that exhibited a two phase separation, clear and aggregate, were not measured.

Soluble protein

After turbidity measurement the samples were clarified by centrifugation at 100,000 × *g* for 2 hr. The protein concentrations of the supernatant solutions were measured by the biuret test (Gornall et al., 1949) or the Folin-Ciocalteu test (Lowry et al., 1951) for concentrations greater than or less than 1 mg/mL, respectively. Bovine serum albumin (Sigma) was used to calculate standard curves.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was done according to the procedure of Laemmli (1970). Separating gels were 10% (w/v) polyacrylamide and 0.267% (w/v) bis-acrylamide; stacking gels were 3.5% (w/v) polyacrylamide and 0.093% (w/v) bis-acrylamide. Gels were stained and destained as outlined by Peng et al. (1982).

RESULTS

A POLYACRYLAMIDE GEL of the protein isolated in separate fibrinogen and myosin isolations is shown in Fig. 1. Myosin preparations always contained some C-protein and M-protein, but no band corresponding to actin was ever seen. Fibrinogen contained slight amounts of contaminating protein. The polyacrylamide gel demonstrated that the standard isolation procedures removed the majority of contaminating proteins.

Fig 2 shows the solubility and turbidity of myosin, fibrinogen and albumin heated singly in 6 mg/mL suspensions at 12°C/hr. Myosin solubility continually decreased between 20°C and 45°C, with the greatest decrease occurring between 41°C and 44°C. Fibrinogen did not decrease in solubility between 20°C and 45°C, but demonstrated a rapid decrease between 46°C and 50°C. Heating albumin to 50°C did not decrease solubility. Albumin began to lose solubility between 50°C and 70°C and then exhibited a major decrease between 70°C and 80°C. The turbidity of all three protein suspensions showed a major increase corresponding to the primary loss of solubility. Turbidity increased more rapidly for fibrinogen than for myosin (Fig. 2).

The effect of heating rate on turbidity increases in myosin and albumin suspensions is shown in Fig. 3. Constant heating appeared to produce the greatest turbidity, while heating at a rate of 50°C/hr produced the least turbidity. This was true for both the myosin and albumin suspensions.

The effect of heating rate on myosin gelation is presented in Fig. 4. In the results of Fig. 2 and 4, it was observed that myosin solubility decreased to a major extent by 44°C, but

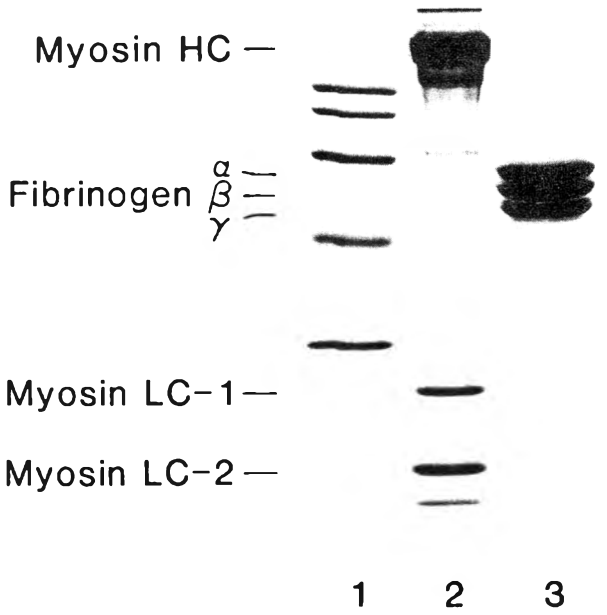

Fig. 1—SDS polyacrylamide gel of purified myosin and fibrinogen. Thirty micrograms of proteins were placed in each well. Lane 1 contains molecular weight standards (daltons) — beta galactosidase (116,000), phosphorylase B (97,400), bovine albumin (66,000), egg albumin (45,000) and carbonic anhydrase (29,000). Lanes 2 and 3 show proteins from typical myosin (2) and fibrinogen (3) isolations.

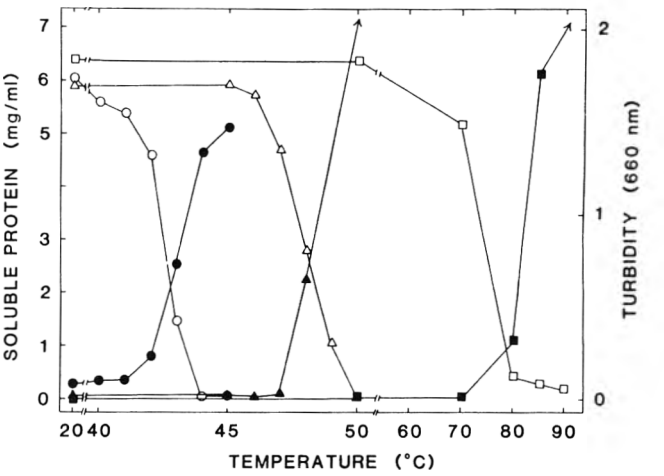

Fig. 2—Turbidity (closed symbols) and solubility (open symbols) for myosin (circles), fibrinogen (triangles) and albumin (squares) suspensions as a function of temperature when heating was done by a linearly increasing rate at 12°C/hr. → Indicates that $A_{660} > 2.0$.

substantial gel strength did not develop until 55°C. The turbidity increase corresponding with the solubility decrease indicated that protein aggregation had occurred but had not yet developed into a gel network. The heating rate had an influence on myosin gel strength. Gels formed at both linear heating rates increased in rigidity between 50°C and 70°C, whereas gels formed under constant heating tended to decrease in rigidity at higher temperatures (Fig. 4). For myosin heated to 70°C at either concentration (Fig. 4 and 6), the 12°C/hr rate produced the most rigid gels.

The strengths of thermally produced fibrinogen gels are reported in Fig. 5 and 6. As was seen in the results for myosin, the linearly increasing heating rates produced the strongest gels at temperatures of 55°C and above. The fibrinogen gels formed

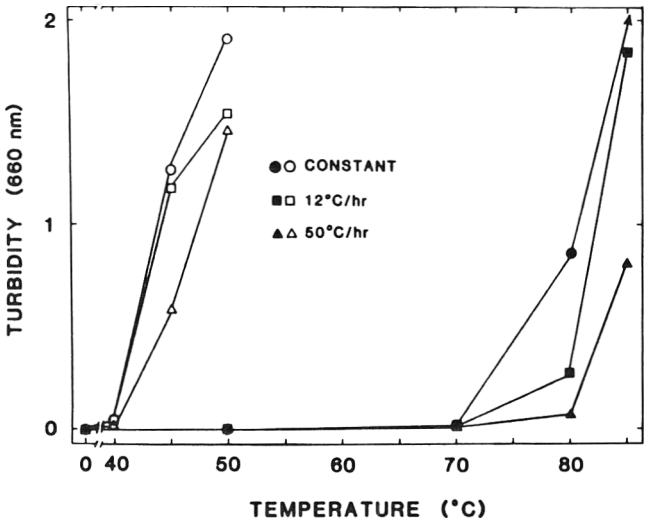

Fig. 3—Turbidity changes in heated myosin (3 mg/ml) (open symbols) and albumin (6 mg/mL) (closed symbols) as a function of temperature for suspensions heated constantly (circles), 12°C/hr (squares) and 50°C/hr (triangles). → Indicates that $A_{660} > 2.0$.

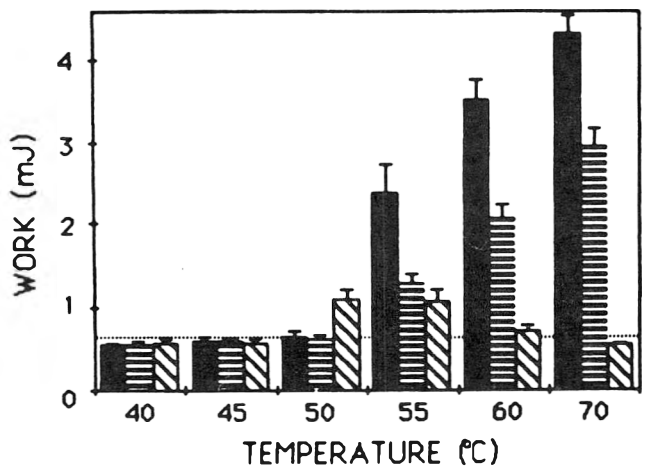

Fig. 4—Myosin (3 mg/mL) gel strength as a function of temperature as developed by constant heating for 20 min (diagonal lines) and at linear rates of 12°C/hr (solid) and 50°C/hr (horizontal lines). Mean values plus standard error bars. Treatments which fall below dotted line were not gelled. Line is average plus two S.D. of 41 samples.

by heating to 70°C at 12°C/hr were much weaker than the myosin gels formed under the same heating conditions. The constant heating conditions decreased the strengths of 3 mg/mL fibrinogen gels between 50°C and 70°C, and the 6 mg/mL gels between 55°C and 70°C.

Albumin gel strengths are shown in Fig. 6. Gelation began to occur in albumin after 80°C and continued to increase to the final sampling temperature of 95°C. Under constant heating, the maximum gel strength for albumin developed at 95°C. There was no major difference between the 12°C/hr and constant heat treatments on the formation of albumin gels. This was in contrast to the differences in the effect of these treatments on both myosin and fibrinogen gels.

DISCUSSION

THE EFFECT of heating rate on turbidity appeared to be both time and temperature dependent. The turbidity of protein heated at 50°C/hr appeared to trail behind the turbidity of protein heated at 12°C/hr. For any sampling temperature, the protein

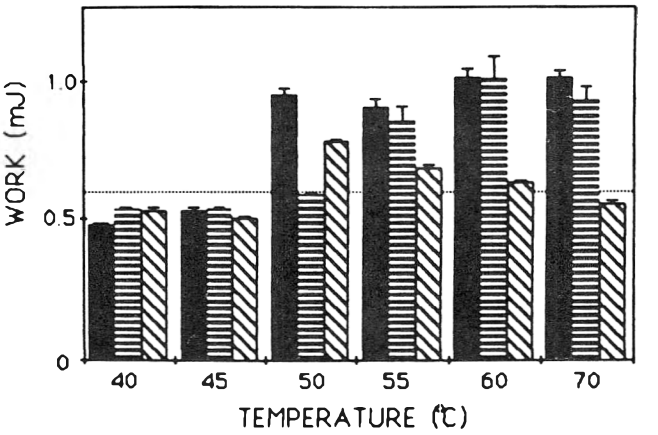

Fig. 5—Fibrinogen (3 mg/mL) gel strength as a function of temperature as developed by constant heating for 20 min (diagonal lines) and at linear rates of 12°C/hr (solid) and 50°C/hr (horizontal lines). Mean values plus standard error bars. Treatments which fall below dotted line were not gelled. Line is average plus two S.D. of 41 samples.

heated at temperatures increasing at 50°C/hr had spent less time above the denaturation temperature than did similar protein heated at 12°C/hr. The greater amount of time in a denatured state could have allowed for more aggregation to occur. Hegg et al. (1978) heated conalbumin suspensions at 18°C/hr, 60°C/hr and 75°C/hr and found results similar to those reported here in that the slowest heating rate produced the greatest turbidity at any given temperature.

The rapid loss of myosin solubility between 41°C and 44°C was due to thermal denaturation and coagulation. This range corresponds well with values for thermal transitions in the rod portion of myosin reported by other workers, with the exact temperature being dependent on pH, ionic environment and method of detecting denaturation (Jacobson and Henderson, 1973; Goodno and Swenson, 1974, 1975a, b; Samejima et al., 1976; Wright et al., 1977; Goodno et al., 1976). Other workers have reported myosin aggregation at 25°C (Lowey and Holtzer, 1959), 30°C (Yasui et al., 1960; Nakayama and Sato, 1971a) and 35°C (Kawakami et al., 1971; Kimura et al., 1980; Penny, 1967). In this study we also detected increases in turbidity between 20°C and 40°C. The turbidity increase in this temperature range was low in magnitude because measurement of turbidity at 660 nm was used to optimize detection of large aggregates formed at higher temperatures.

Fibrinogen has been shown to have two pH dependent thermal transitions when analyzed by differential scanning calorimetry (Donovan and Mihalyi, 1974; Medved et al., 1981). The initial melt occurred between 45°C and 61°C, and the second between 89°C and 100°C (Donovan and Mihalyi, 1974; Medved et al., 1981). Thus, loss of solubility and turbidity increase observed in this study appeared to correlate well with the first thermal transition seen in calorimetry studies. The decreased albumin solubility between 50°C and 70°C also agrees with other reports. Harmsen and Braam (1969) reported aggregation at 60°C when bovine serum albumin as heated at 250 mg/mL in a pH 7.0 buffer, and Shimada and Matsushita (1981) showed that initial turbidity developed between 60°C and 65°C in a 280 mg/mL, pH 6.5 solution of bovine serum albumin. Albumin solubility at 70°C was 81% of the unheated solution while both fibrinogen and myosin were at <0.2% of their original solubility. Thus in processed meats where normal endpoint temperature seldom exceeds 70°C, a large percentage of the albumin could be in the soluble state.

The data presented in Fig. 4, 5, and 6 demonstrate that an increase in protein concentration caused an increase in gel strength. These data are in agreement with other studies con-

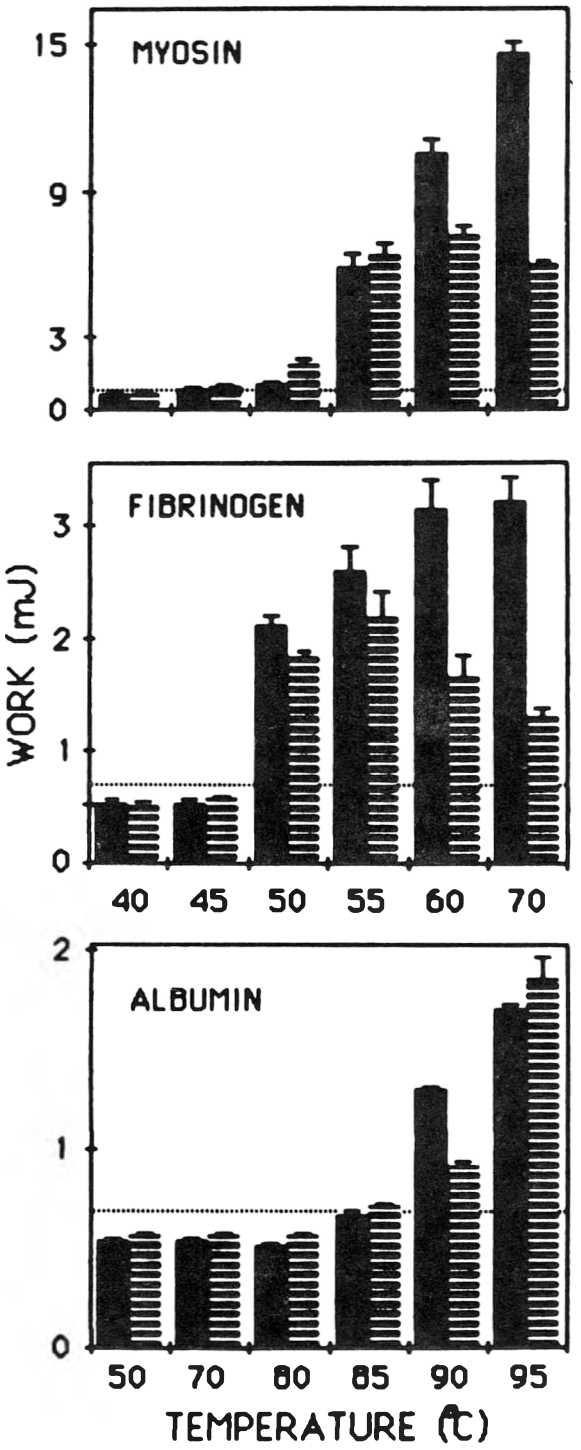

Fig. 6—Gel strength developed by heating proteins (6 mg/mL) for 20 min (horizontal lines) and at a linear rate of 12°C/hr (solid). Treatments which fall below dotted lines were not gelled. Lines are average plus two S.D. of 31 samples.

cerning myosin gelation (Ishioroshi et al., 1979). The heating rate had a major effect on the strength of both myosin and fibrinogen gels. Constant heating at 70°C of both proteins at 3 mg/ml did not allow for a gel structure to develop and the cooled suspensions developed a clear upper zone indicating a discontinuous protein phase. The slower heating rates allowed for gelation to occur at 3 mg/mL; thus, it appeared that the constant heating, which resulted in the most rapid temperature rise, favored more random protein-protein interactions rather than ordered interactions which would have led to development of a three-dimensional gel network. A gel did develop when 6 mg/mL suspensions were heated in the constant manner, indicating that both random and ordered protein-protein interactions occurred, but at 3 mg/mL there apparently were not enough ordered interactions to develop a gel skeleton.

Ferry (1948) proposed a two-step mechanism for gelation of denatured proteins. The initial phase being the conversion of native protein to the denatured state, and the second phase being the orientation of denatured protein into a gel network. Hermansson (1978) further defined denatured protein aggregation as "coagulation" when the interaction is random, and "gel forming" when there is order in the aggregation. The main factor driving the denatured protein to coagulation or gel formation is the rate of association, with slower rates favoring gel formation. Our work supports the two-step mechanism to the extent that some thermal alteration in the protein structure must precede association into a gel structure. Myosin gels formed at both protein concentrations by linear heating rates continued to increase in strength between 50°C and 70°C. This coincides with thermal denaturation studies that indicate the supercoiled α-helix tail portion of myosin unfolds slowly as temperature increases between 30°C and 70°C (Burke et al., 1973; Samejima et al., 1976; Tsong et al., 1979). Our data, viewed together with that of Burke et al. (1973), suggests that a myosin molecule with 32% α-helix remaining (55°C) produces a weaker gel network than one with 4% α-helix remaining (70°C). Thus, it appears that thermal alteration of myosin and its association into a gel network occurs continuously between 55°C and 70°C.

Albumin gelation was not affected by heating rate to the extent seen in fibrinogen and myosin gelation. In fact there appeared to be no difference between the 12°C/hr and constant heating treatments. The trends in albumin gelation at 95°C appeared quite similar to those observed for 6 mg/mL of myosin at 55°C and 6 mg/mL of fibrinogen at 50°C. This suggests that only the initial phase of albumin gelation was observed, and if heating had been continued to elevated temperatures similar rate effects may have been seen. Work by Tombs (1970) supports this speculation by showing that constant heating of a 10 mg/mL solution of bovine serum albumin, buffered at pH 6.24 produced a gel at 70°C and a coagulate at 100°C.

At a protein concentration of 6 mg/ml, myosin formed the strongest gels followed by fibrinogen and then albumin. Tombs (1970, 1974) discussed thermally induced protein gels as three-dimensional networks which resulted from random or ordered aggregation. If one assumes that a minimum network is needed to form a gel, then there would be both a minimum length to that network and, depending on the size of the molecular building block, a minimum number of molecules. Using 6 mg/mL solutions of the three proteins studied in these experiments and molecular dimensions of the native proteins, myosin, fibrinogen and albumin would contribute lengths of 1.0×10^9, 4.8×10^8, and 1.5×10^9 meters, respectively (Squire et al., 1968; Williams, 1981; Lowey et al., 1969). These lengths are not in agreement with gel strength results, which is probably because these values are for the native protein states whereas the gels were formed with denatured molecules. Hence, the degree of unfolding and type of gel matrix formed are important. The myosin gelation data are in agreement with this concept because, as previously stated, myosin continues to unfold in the tail region between 30°C and 70°C with a corresponding increase in gel strength. The previously discussed point does not explain why the constant heating treatments decreased myosin and fibrinogen gel strengths at elevated temperatures. Possibly, once the proteins were thermally altered, gel formation proceeded according to a nucleation and growth process. Faster heating rates would favor more nucleation and less growth; thus constant heating at temperatures substantially above thermal denaturation would produce more nuclei and allow less growth to occur. This concept is supported by Tombs (1970). Using electron microscopy he observed that bovine serum albumin heated at 70°C formed a gel which appeared as chains, but when this protein was heated at 100°C a coagulate formed

which appeared to be a combination of chains and spheres. A more extensive kinetic analysis would be needed before the nucleation/growth model could be fully applied.

Long time–low temperature cookery is used extensively in the meat industry because it decreases moisture loss and increases tenderness. The tenderness increase has appeared to be due to action of endogenous proteases while the decrease in moisture loss has not been fully explained. Data presented in the study suggest that a slow heating rate may allow for more favorable protein-protein interactions to occur and thus decrease the moisture loss. The differences in gelation temperature and gel strength between albumin and fibrinogen demonstrate that individual plasma proteins vary widely in protein functionality. The fractionation of blood plasma proteins into isolates based upon protein functionality would broaden and improve the food product applications of blood plasma proteins.

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Interaction of Myosin-Albumin and Myosin-Fibrinogen to Form Protein Gels

E.A. FOEGEDING, W.R. DAYTON, and C.E. ALLEN

ABSTRACT

Myosin, fibrinogen, albumin, myosin-fibrinogen and myosin-albumin gels were formed by heating in pH 6.0 phosphate buffer at two heating rates. Gel strength was measured with an annular pump and soluble protein was determined. Myosin and fibrinogen interacted to form a gel which was stronger than the sum of the gel strengths for the two individual proteins. The strength of myosin-fibrinogen gels formed at 50°C was not affected by heating method; however, the strength of gels developed between 55°C and 70°C was related to heating method. Myosin and albumin did not interact to form a gel matrix until 80°C where sufficient thermal alteration of albumin had occurred.

INTRODUCTION

NONMEAT PROTEINS are generally added to meat systems with a specific function in mind. If one wanted to improve emulsion stability, a protein known to stabilize emulsions would be added. In performing a functional role, such as emulsion stabilization, a protein can act independently of the meat emulsifying proteins or it could interact with them in an additive or synergistic manner. Johnson and Zabik (1981) reported that egg albumin proteins behaved differently when heated in combination compared to heating alone. Knapp et al. (1978) reported that the globin/plasma protein ratio affected the texture of heat processed emulsions.

Plasma contains ca 150 different proteins (Andersson and Lunden, 1979) that vary greatly in size and shape. The ability to function in a food system varies among the plasma proteins, with albumin having better foaming characteristics and globulins performing better as emulsifiers (Pentaedo et al., 1979). Thermally induced gelation is another functional property of food proteins and bovine plasma has been shown to gel upon heating (Harper et al., 1978; Comer, 1979; Hickson et al., 1980, 1982).

In a previous study we investigated the effect of heating rate on thermally induced gelation of bovine serum albumin, fibrinogen and porcine myosin (Foegeding et al., 1986). The objectives of this study were to investigate the gelation of combinations of myosin-albumin and myosin-fibrinogen, and to determine whether heating rate had an effect on the formation of gels from those combinations.

MATERIALS & METHODS

BOVINE SERUM ALBUMIN (fatty-acid free) was obtained from Sigma Chemical Company (St. Louis, MO). Myosin and fibrinogen were isolated as described previously (Foegeding et al., 1986). Protein mixtures were made by adjusting individual proteins to concentrations twice the level desired in the mixture. The two protein suspensions were combined in a 1:1 ratio and mixed for ca 10 min at 4°C prior to being portioned into 16 × 100 mm disposable culture tubes, as described by Foegeding et al. (1986). All other procedures used in this study have been described in the preceding paper (Foegeding et al., 1986). Briefly, protein suspensions were heated either at a linearly increasing rate of 12°C/hr or in a water bath at the desired temperature. Duplicate samples were removed from heating, held at 4°C overnight, then tested for gel strength, tubidity and soluble protein. Significant differences between means were determined by using the Student's t-test (Steel and Torrie, 1980). Each treatment had two replications.

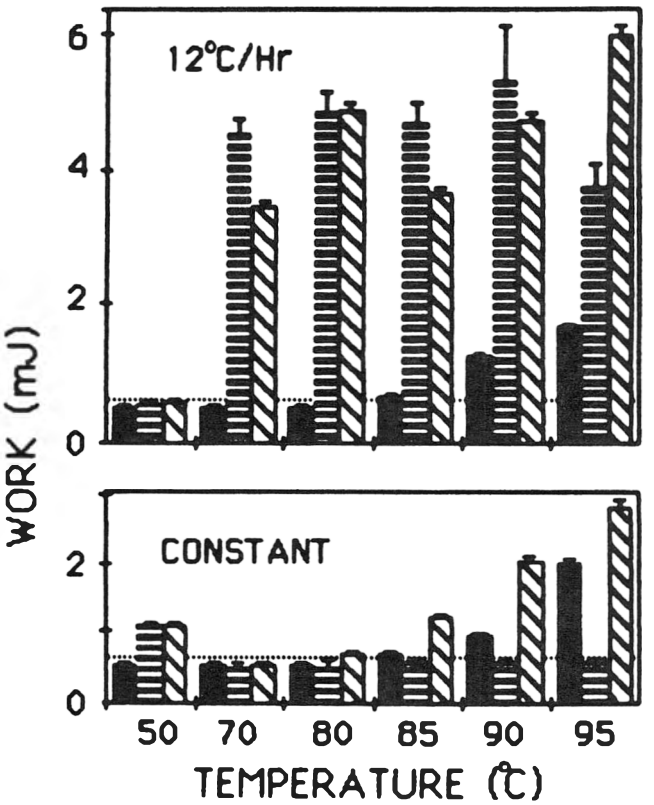

Fig. 1—Gel strength developed in myosin (3 mg/mL horizontal lines), albumin (6 mg/mL, solid) and myosin-albumin (3 mg/mL-6 mg/mL, diagonal lines) suspensions by constant heating for 20 min and at a rate of 12°C/hr. Mean values plus standard error bars. Treatments which fall below dotted lines were not gelled. Lines are average plus two S.D. of 41 samples.

RESULTS & DISCUSSION

Myosin-albumin

Figure 1 illustrates the gel strength developed when suspensions of myosin, albumin or myosin-albumin were heated at a linear rate of 12°C/hr or at a constant temperature for 20 min. Myosin was heated at 3 mg/mL because at that concentration gelation was extremely dependent on the heating conditions. Albumin was heated at 6 mg/mL because that appeared to be the minimum protein concentration for gelation in the buffer

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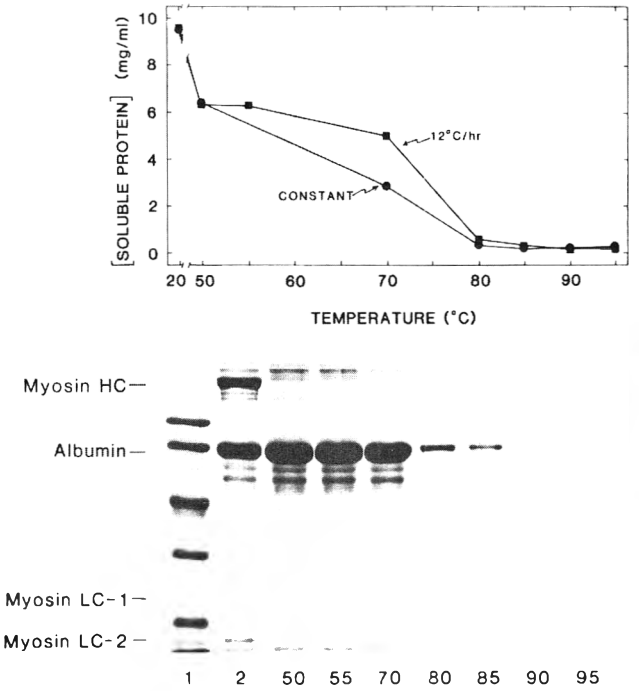

Fig. 2—Top. Effect of heating rate on protein solubility of myosin-albumin (3 mg/mL-6 mg/mL). Bottom. SDS-polyacrylamide gel of protein remaining soluble after heating myosin albumin (3 mg/mL-6 mg/mL) at 12°C/hr from 20°C to 95°C. Lane 1 — molecular weight standards; lane 2 — 45 μg unheated myosin-albumin mixture. Lanes 3–9 contained 6.6 μL of soluble protein solutions after heating to the temperature indicated.

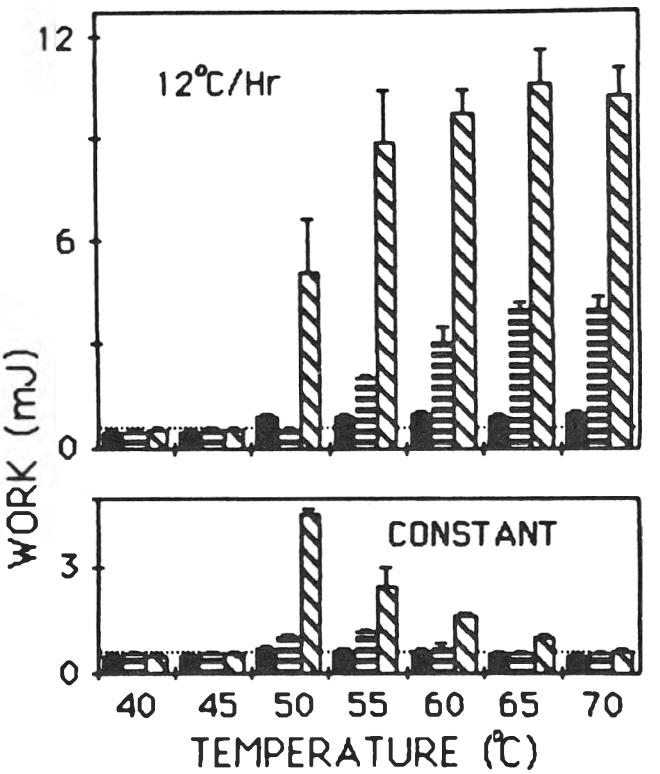

Fig. 3—Gel strength developed in myosin (3 mg/mL, horizontal lines), fibrinogen (3 mg/mL, solid) and myosin-fibrinogen (3 mg/mL-3 mg/mL, diagonal lines), suspensions by constant heating for 20 min and at a rate of 12°C/hr. Mean values plus standard error bars. Treatments which fall below dotted lines were not gelled. Lines are average plus two S.D. of 41 samples.

used in this study. There were few data points taken below 80°C because in our buffer albumin did not gel below 80°C.

These data support our previous findings (Foegeding et al., 1986) that a linear heating rate produced stronger gels than did constant heating. Myosin (3 mg/mL) heated at 12°C/hr formed strong gels while myosin heated at constant temperatures failed to gel between 70° and 95°C (Fig. 1). The heating rate did not show a major effect on albumin gelation. Mixtures of myosin and albumin formed stronger gels with linear compared to constant heating. In comparing the gels formed by linear heating of myosin or myosin-albumin suspensions, no consistent trend was found. The myosin-albumin mixture consistently formed the strongest gels among constant heating comparisons at 85°, 90°, and 95°C. It appeared that the gels formed by this mixture at constant temperatures of 85°, 90°, and 95°C had some contribution from myosin. It should be noted that myosin, if heated alone, would not gel under these conditions (Fig. 1). Constant heating of the myosin-albumin mixture at 70°C did not produce a gel; thus it appears that albumin must be thermally altered before it can interact with myosin in the mixture. The requirement for both proteins to be thermally altered prior to interacting is supported by Peng et al. (1982a, b) who showed that soybean 11S protein had to be thermally altered before it could interact with myosin. The high temperature requirement and lack of sensitivity to heating rate suggests that rapid heating situations, such as boil-in-the-bag meats, may be a favorable application for serum albumin.

The species of protein that remained soluble after heating at 12°C/hr are shown in Fig. 2. Myosin heavy chain and light chain 2 showed major losses in solubility after heating to 50°C. Light chain 1 decreased in solubility as heating progressed, but a band was still detectable after heating to 95°C. The albumin band remained relatively constant between 50°C and 70°C then decreased at 80°C and continued to decrease to 95°C. Myoglobin was shown to be the only meat protein that remained soluble at a detectable level when beef was heated to 85°C (Lee et al., 1974); thus the solubility of albumin at 85°C could be investigated as a means to detect its presence in meat. The major decrease in solubility for both myosin heavy chain and albumin preceeded gel formation. The protein concentration curve (Fig. 2) shows that at 70°C the solubility of myosin-albumin was decreased more by constant heating than by heating at 12°C/hr. Polyacrylamide gels (not shown) indicated that this decrease in solubility at 70°C (constant heating) was due to a loss of albumin. Although Fig. 2 shows minor protein bands above myosin heavy chain, there was no significant change in these bands with heating, thus no soluble protein complexes were formed via nondisulfide covalent bonding.

Myosin-fibrinogen

The effects of heating rate on myosin, fibrinogen and myosin-fibrinogen gelation are presented in Fig. 3. At 3 mg/mL both myosin and fibrinogen gelation were extremely dependent on heating rate. The myosin-fibrinogen gels formed at 12°C/hr were stronger than the sum of the gel strengths produced by the individual proteins at the same heating rate. Thus, it appeared that myosin and fibrinogen interacted to form a gel matrix when heated to temperatures (60–70°C) normally encountered in meat processing. Furthermore, myosin and fibrinogen heated at 12°C/hr interacted to produce gels that were stronger than myosin-albumin gels and that gel formation occurred at lower temperatures and protein concentrations. However, heating at constant temperatures produced substantially different results (Fig. 3). Constant heating of myosin-fibrinogen produced maximal gel strength at 50°C; then, like the individual proteins, gel strength decreased as heating temperature increased. The gel strength for myosin-fibrinogen gels formed by constant heating at 50°C was much greater than for the individual proteins. The heating rates tested had minimal effect on the strength of gels formed at 50°C; then, like the individual proteins, gel strength decreased as heating temper-

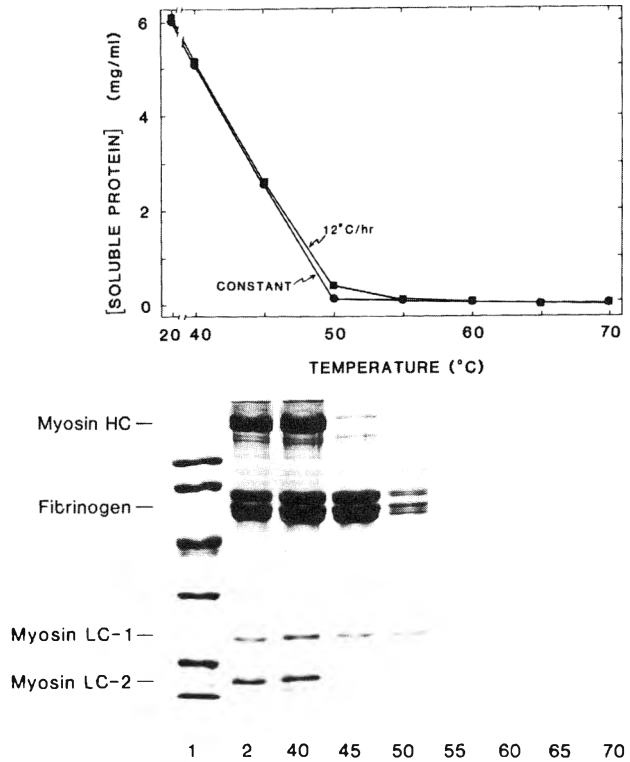

Fig. 4—Top. Effect of heating rate on protein solubility of myosin-fibrinogen (3 mg/mL-3 mg/mL). Bottom. SDS-polyacrylamide gel of protein remaining soluble after heating myosin-fibrinogen (3 mg/mL-3 mg/mL) at 12°C/hr from 20°C to 70°C. Lane 1 — molecular weight standards; lane 2 — 60 μg unheated myosin-fibrinogen mixture. Lanes 3–9 contained 13.2 μL of soluble protein solutions after heating to the temperature indicated.

ature increased. Between 55°C and 70°C gel strength was significantly affected by heating rate. In the previous paper (Foegeding et al., 1986) it was suggested that rapid heating favored random protein-protein interaction and slow heating allowed for a more ordered interaction. This would explain the results for gels formed between 55°C and 70°C, but it fails to explain why differences due to heating rate were not seen at 50°C.

The quantitative and qualitative decreases in protein solubility of myosin-fibrinogen suspensions heated at 12°C/hr are shown in Fig. 4. The polyacrylamide gel (Fig. 4) shows that myosin heavy chain and fibrinogen decreased in solubility between 40–45°C and 45–50°C, respectively. The major increase in gel strength between 50°C and 70°C (Fig. 3) correlated with almost a total loss of protein solubility, although myosin light chain 1 remained soluble (Fig. 4). This decreased solubility was also observed in the heated myosin-albumin mixture (Fig. 2). The loss of protein solubility due to heating was similar when suspensions were heated at 12°C/hr or at a constant temperature for 20 min (Fig. 4).

In an attempt to further characterize myosin-fibrinogen gelation, myosin and fibrinogen were combined in concentrations where myosin could gel individually and fibrinogen could not develop measurable gel strength. The results are shown in Table 1. At 50°C both myosin-fibrinogen combinations produced significantly ($P < 0.01$) stronger gels than equivalent concentrations of myosin. As the temperature increased, the myosin suspension progressively formed stronger gels, but did not exceed that of the myosin-fibrinogen mixture until heated to 65°C (Table 1, experiment 2). The greater gel strength of the myosin-fibrinogen mixture compared to myosin alone at 50°C appeared to be due to interaction effects of a myosin-fibrinogen complex.

The rate of heating did not affect myosin-fibrinogen gelation

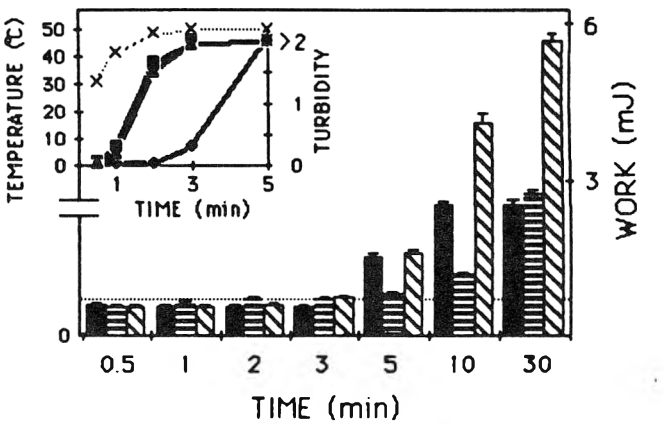

Fig. 5—Gel strength and turbidity ($A_{660\ nm}$ insert) developed by myosin (6 mg/mL, squares and horizontal lines), fibrinogen (6 mg/mL, circles and solid bars) and myosin-fibrinogen (3 mg/mL-3 mg/mL, triangles and diagonal lines) suspensions after constant heating at 50°C. Temperature (x) of buffer in 16 × 100 mm culture tubes at various times after being placed in a water bath at 50°C.

Table 1—Gel strength (mJ) developed in protein suspensions of equal concentration containing myosin or myosin + fibrinogen heated to 50, 55 or 65°C at 12°C/hr

	Final temperature achieved		
	50°C	55°C	65°C
Experiment 1			
4.5 mg/mL myosin	0.86	4.43	7.72
3 mg/ml myosin + 1.5 mg/mL fibrinogen	3.19	5.95	6.63
Level of significance[a]	P<0.01	P<0.01	N.S.[b]
Experiment 2			
3.75 mg/mL myosin	0.78	3.64	6.73
3 mg/mL myosin + 0.75 mg/mL fibrinogen	1.72	4.17	5.39
Level of significance	P<0.01	N.S.	P<0.01

[a] Significant differences between means in column determined by the student's t-test (Steel and Torrie, 1980).
[b] N.S. = not significant (P>0.05).

up to 50°C (Fig. 3). To resolve the initiation of gel formation, an experiment was run to study transitions that occur at various time intervals during 50°C constant heating. The results are shown in Fig. 5. The heating temperature curve is for 5 mL of buffer heated in 16 × 100 mm tubes. Turbidity curves indicated that aggregation occurred prior to gelation. There was more of a delay between aggregation and gelation for myosin than for fibrinogen. Fibrinogen began aggregating later than myosin but developed gel strength faster than myosin alone. A maximal gel strength was observed in fibrinogen by 10 min, while myosin gel strength increased for up to 30 min of constant heating. Thus, it appeared that rate of aggregation into a gel matrix was more rapid for fibrinogen than myosin. In the 3 to 5 min time interval (Fig. 5), the myosin-fibrinogen mixture showed trends similar to fibrinogen gelation. In the 10 to 30 min interval, the mixture behaved in a manner similar to myosin alone. At equal protein concentrations the mixture formed gels stronger than for either individual protein at 10 and 30 min.

Physicochemical aspects of thermally induced protein gelation were discussed by Hermansson (1978) and those concepts were used in deriving the outline shown in Fig. 6. In our study the heating rate up to, and slightly past, the initial point of thermal denaturation did not affect gel formation in the protein mixture. The heating rate and/or time of heating during the aggregation process appears to influence whether an ordered protein gel or an unordered coagulate will be formed at higher temperatures when heating myosin or myosin-fibrinogen at low concentrations (Reaction 3 in Fig. 6). Assuming

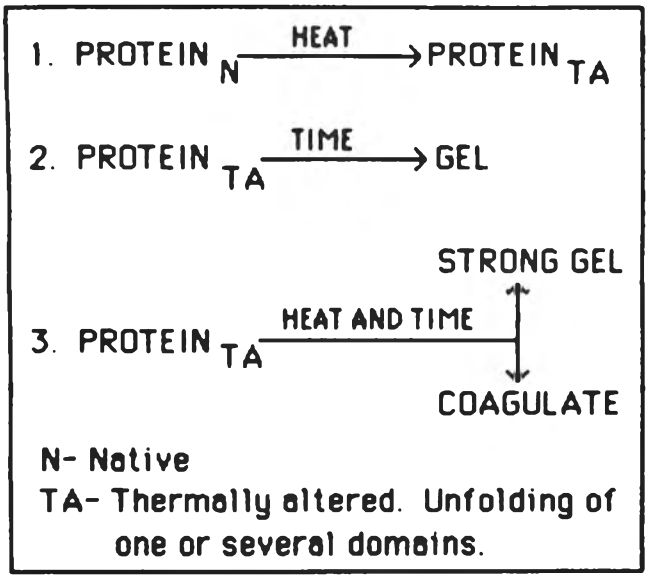

Fig. 6—Proposed events in thermally induced protein gelation.

that there is not a complete partitioning into either a coagulate or gel (Fig. 6), the quantity of protein present in each aggregate would affect gel strength. The time element could be viewed as a requirement for the proteins to associate into an ordered structure and/or allow for greater unfolding of the myosin molecule, as shown by Yasui et al. (1971). In our study the myosin and myosin-fibrinogen gels formed by constant heating all had similar time for association, thus the decrease in gel strength was not related to association rate. This suggested that the variation was due to the degree of unfolding. Data trends in Table 1 could be due to an unfolding of the myosin tail between 50° and 65°C thereby resulting in an increase in the length of protein available to form a gel matrix. Calorimetric investigations have showed that myosin has several unfolding domains, with transitions of 43, 48, 50, 51, 56, and 61°C (Potekhin and Privalov, 1979). The effective length added to the gel network per myosin molecule would then increase as temperature increases. Variation in the gel network, such as the degree of crosslinking and chemical bonding, could also account for the observed variations in gel strength.

In conclusion, this study demonstrated that combinations of myosin and fibrinogen form stronger gels than myosin-albumin combinations. The effect of heating rate on gel strength was dependent upon the combination of proteins and the end-point temperature of the protein gel.

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Prediction of Thermal Conductivity of Vegetable Foods by the Effective Medium Theory

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ABSTRACT

The Effective Medium Theory (EMT), known in the field of physics to model conductive properties of heterogeneous chaotic media, has been applied to predict the thermal conductivity of sugar-based fruits and of potatoes as a function of the water content. With the thermal conductivity of the constituents, the composition and porosity as data, the equation derived from the EMT for continuous media was applied to apples, pears, and potatoes. The results when compared with experimental values obtained by the probe method and from the literature showed good agreement, with differences not larger than 8%, though the model appeared to be over predictive, particularly for higher water contents.

INTRODUCTION

A VEGETABLE FOOD TISSUE can be physically regarded as a composite, heterogeneous medium, made up of a continuous matrix of cells into which a network of pores is dispersed at random. Processing of these products usually involves heat and mass transfer through such a medium, making transport properties, like thermal conductivity, relevant parameters. Effective thermal conductivities must either be measured or predicted from the constituents' individual properties and their physical arrangement. A dependable fully predictive method is of particular interest in design, optimization and process analysis applications.

For many years authors have produced models to predict the effective thermal conductivity of heterogeneous materials, such as porous media, suspensions and solid mixtures, either theoretical or semi-empirical in nature. Progelhof et al. (1976) have made a comprehensive review of different predictive methods.

In all cases it has been assumed that the medium is composed of two phases, one continuous and the other one disperse, of thermal conductivities k_c and k_d, respectively, a certain fraction ϵ_d of the volume occupied by the latter. Assuming a given space distribution or topological arrangement of the phases, as well as shape (sphere, rods) of the disperse phase, several correlations for the effective property k_m have been proposed as a function of k_c, k_d and ϵ_d.

When drying of a fruit or vegetable is studied, the changing moisture content is an important variable, due to its contribution to the property values and because the physical structure is directly related to it. Though some data can be found on thermal conductivity of foodstuffs of different water content (Sweat, 1974; Rha, 1975), they refer to fresh product moisture contents rather than to different values associated with decreasing moisture. One exception is the work by Lozano et al. (1979).

Landauer (1952) proposed an analytical approach to the prediction of thermal or electrical conductivity of heterogeneous chaotic media, constituted by two or more constituents distributed at random. The basic idea is to assume the existence of a virtual, homogenous medium, called effective medium, with a conductivity k_m equal to the overall conductivity of the actual system. Local perturbations introduced by the presence of non-homogenities are treated as fluctuation of the effective medium and the derivation is called the Effective Medium Theory (EMT).

The objective of this study was to adapt the EMT theory to the prediction of effective thermal conductivities of vegetable foodstuffs undergoing drying. Easy to apply, the theory has produced good results in comparison with experimental values, its main feature being that only k_c, k_d, and ϵ_d are required, regardless of the geometrical structure.

Theory

Landauer (1952) and Davis et al. (1975), by different reasoning, arrived at the same expression for a medium with n constituents:

$$\sum_{i=1}^{n} \epsilon_i \frac{k_m - k_i}{k_i + 2\,k_m} = 0 \qquad (1)$$

where ϵ_i and k_i are the volume fraction and thermal conductivity of the i-th constituent.

Kirkpatrick (1973) extended the EMT to regular networks of resistors distributed at random, obtaining a similar equation

$$\sum_{i=1}^{n} \epsilon_i \frac{k_m - k_i}{k_i + \left(\frac{Z}{2} - 1\right) k_m} = 0 \qquad (2)$$

where Z, the coordination number of the system, is the number of conductors with a site in common. Kirkpatrick (1973) assumed that the continuous medium can be represented by a cubic arrangement, for which Z = 6, such that Eq. (2) becomes Eq. (1).

For a system of two components, with k_1 and $k_2 \neq 0$, Eq. (2) can be written:

$$\frac{k_m}{k_2} = b + \left\{b^2 + \frac{2p}{Z - 2}\right\}^{0.5} \qquad (3)$$

where $p = k_1/k_2$

and

$$b = \frac{(Z.\epsilon_2/2) - 1 + p\,\{(Z/2)\,(1 - \epsilon_2) - 1\}}{Z - 2} \qquad (4)$$

Natural solid foodstuffs are basically cellular tissues in which Z should represent the number of cells in contact with a given cell. Assuming a cubic arrangement it would be Z = 6, which is the value corresponding to the continuous media. In this sense, the porous tissue is regarded as a homogeneous substance.

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MATERIALS & METHODS

Thermal conductivity measurements

For pears and potatoes no previous experimental data were available and it was obtained as follows: Cylindrical samples, 55 mm long and 16 mm in diameter, were cut from Williams pears and Spunta potatoes, previously kept in cold storage (4–6°C).

A set of six samples was partially dried in a hot air drier, at 55°C, time being the control variable to obtain a given moisture, wrapped with polythelene film, kept in cold storage for 24 hr to obtain uniform moisture distribution and finally kept at test temperature (20°C) for 5 hr to flatten the temperature distribution profile.

Thermal conductivity was measured on three samples by the probe method, as described by Lozano et al. (1979), the remainder being used to determine moisture content at 70°C under vacuum. All replications were averaged. Moisture content varied from that of the fresh product down to X = 0.5; 64 points at fairly even intervals were obtained for each product. Below X = 2, samples were cut in parallelepipeds of 25 mm × 25 mm × 60 mm to take into account shrinking effects.

RESULTS & DISCUSSIONS

FROM THE THEORETICAL considerations above, it is apparent that application of EMT calls for knowledge of constituents' thermal conductivities, k_i, and volume fractions, ϵ_i. These data are either experimental or estimated. In what follows some estimation methods are suggested for those cases in which no data are available. It is also shown that, depending on the composition and the degree of heterogeneity of the material, it is enough to use the EMT only once, as in apples and pears, or it can be used repeatedly, to build up the required k_i data as in the case of potatoes.

In all cases, the cellular matrix is regarded as the continuous phase, in which pores (intercellular air spaces) are dispersed at random. Consequently, k_c will be that of the cellular material, while k_d will be that of the air. Pores' volume fraction, ϵ_d, will be simply the total porosity, ϵ, and the cellular fraction, $\epsilon_c = 1 - \epsilon$.

Apples and pears

The cells can be assumed to be cellulose capsules containing sugar solutions of variable concentration in accordance with the dehydration level. Since soluble solids are mainly reducing sugars, the thermal conductivity of the continous phase was approximated by that of the glucose solutions. This was obtained by correlating experimental data from Riedel (1949), using a least square fit. To tie this result to moisture content, it is necessary to relate the sugar/dry matter ratio, σ, of each fruit, to the water/dry matter ratio, X. Introducing this value into the correlation obtained, the cell conductivity is predicted by:

$$k_{cell} = 0.5981 - 0.3165 \frac{\sigma}{\sigma + X} \text{ (at 20°C)} \qquad (5)$$

where $\sigma = 0.76$ for apples (Rotstein and Cornish, 1978) and $\sigma = 0.83$ for pears (Watt and Merrill, 1963).

Eq. (5) takes into account the contribution of water and sugar only, since the cellulose can be neglected due to its low concentration. The first term in the right hand side of Eq. (5) is the contribution of water and the second one represents the influence of sugar in the conductivity.

Thermal conductivity of air is $k_d = 0.0254$ W/m°C, as reported by Batchelor (1967). Total porosities were measured and correlated by Lozano et al. (1980, 1983) as a function of the moisture content. Eq. (6) and (7) correspond to apples and pears respectively.

$$\epsilon = 1 - \frac{0.852 - 0.462 \exp(-0.66\,X)}{1.54 \exp(-0.051\,X)} \qquad (6)$$

and

$$\epsilon = 1 - \frac{1.251 - 0.022X - 0.107 \exp(-1.89 \times 10^{-7}X)}{0.832 + 0.031X + 0.632 \exp(-0.395\,X)} \qquad (7)$$

for $0 < X < 8$.

Potatoes

The potato tissue shows distinctive differences from that of sugar-based fruits. While in the latter the cellular matrix is assumed to be represented by a sucrose solution of variable concentration, the potato cell can be described as a suspension of starch granules in a very dilute aqueous solution (Nara, 1979). However, the starch granule is in turn a nonhomogeneous material, since it consists of starch saturated with adsorbed water. Therefore, the medium thermal conductivity of the material requires successive applications of the EMT to the starch granule, to the granules suspension and, finally, to the porous cellular tissue-air system.

The starch granule can be assumed to be constantly saturated for all practical values of the cellular water content, since the saturation value is $X_s = 0.49$g H_2O/g dry starch. The volumetric fraction of dry starch and absorbed water are, respectively:

$$\epsilon_{s,g} = \frac{v_s}{v_s + v_w} = \frac{1/1.519}{1/1.519 + 0.49/1} = 0.5733$$

and

$$\epsilon_{w,g} = 1 - \epsilon_{s,g,} = 0.4267$$

Thermal conductivities at 20°C of dry starch and water are, respectively:

$k_s = 0.216$ W/m°C (Roth and Tsao, 1970)

$k_w = 0.590$ W/m°C (Batchelor, 1967)

Substituting these values in Eq. (3) and (4), the effective thermal conductivity of the saturated starch granule results:

$$k_g = 0.3486 \text{ W/m°C}$$

The volumetric fraction of starch granules and water in the cell are not constant but a function of water content. Assuming that all solids in the cell are starch only, which is consistent with the assumption that the dilute solution is pure water, the fractional volume of water in the cell is given by:

$$\epsilon_{w,c} = \frac{X - 0.49}{(X - 0.49) + (1/\delta_s)} \qquad (8)$$

where δ_s is (Nara, 1979) given by:

$$\delta_s = 1.519 - \frac{X}{3} \text{ g/cm}^3$$

and for $X_s = 0.49$, $\delta_s = 1.356$.

Substituting that value in Eq. (8), it is possible to calculate $\epsilon_{w,c}$ for different values of X, and consequently the volumetric fraction of the starch grain in the cell, $\epsilon_{s,c} = 1 - \epsilon_{w,c}$. With the values of k_w and k_g given above, the medium cell conductivity k_c is calculated at the same X values, by means of equations (3) and (4). Then the average thermal conductivity of the potato, k_m, can be predicted at different water contents, provided that total porosity, i.e. the air volume fraction, is known as a function of X.

Lozano et al.(1983) presented an experimental correlation valid for $0 < X < 4.5$

$$\epsilon_a = 1 - \frac{1.202 - 0.033\,X - 0.2585 \exp(-3.466\,X)}{1.234 - 0.026\,X + 0.085 \exp(-4.231\,X)} \qquad (9)$$

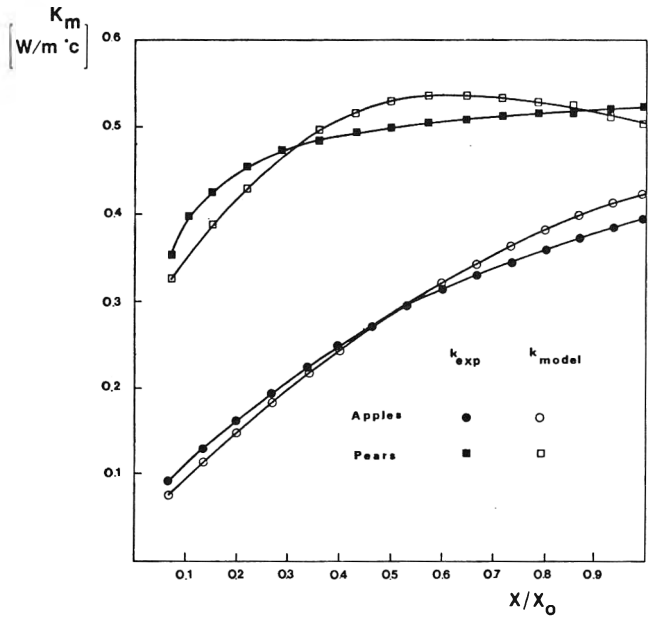

Fig. 1—Experimental and EMT prediction of thermal conductivity of Granny Smith apples and Williams pears.

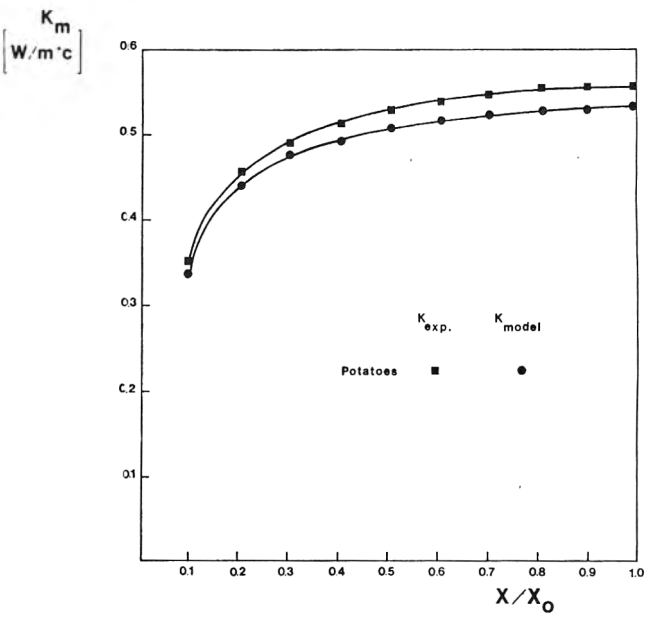

Fig. 2—Experimental and EMT prediction of thermal conductivity of Spunta potatoes.

which was used in Eq. (3) and (4) as ϵ_1, as well as the cell volumetric fraction, $\epsilon_c = \epsilon_2 = 1 - \epsilon_a$, $k_2 = k_c$ calculated above and $k_1 = k_a$.

The predicted curves were tested against experimental ones. For Granny Smith apples, the correlation presented by Lozano et al. (1979) was used:

$$k_m = 0.490 - 0.443 \exp(-0.206\ X)\ W/m°C \quad (10)$$

The measured values for potatoes and pears were correlated by Eq. (11) and (12) respectively.

$$\text{Potatoes: } k_m = 0.5963 - \frac{0.1931}{X} + \frac{0.0301}{X^2} \quad (11)$$

$$\text{Pears: } k_m = 0.4875 - \frac{0.0566}{X} + 0.0227\ \ell n\ (X) \quad (12)$$

both in W/m°C, and with correlation coefficient $r^2 = 0.95$.

In Fig. 1 the theoretical curves for pears and apples are shown with the experimental data represented by Eq. (12) and (10) respectively. Agreement between both curves is apparent for apples along the whole range. Regarding pears, the modelled curve showed a different behavior, but within the water content range of practical value. $2 < X/X_o < 1$, error was lower than 4%. The maximum shown by the theoretical curve at $X/X_o = 0.6$ agrees with the minimum found in total porosity by Lozano et al. (1983), (Eq. 7), showing the sensitivity of the model to porosity.

In Fig. 2 the results for potatoes are shown. The predicted curve showed the same behavior as the experimental curve, though consistently lower. The maximum deviation was shown to be 7.5% at $X/X_o = 1$, decreasing to 5.8% at $X/X_o = 0.2$.

Román et al. (1983) have shown that in modeling sorptional equilibrium a fourfold change in effective thermal conductivity has little effect on the predicted moisture decrease and center temperature decrease with time. When internal control prevails, as in most drying applications, a ± 20% error is acceptable. From this standpoint it is felt that EMT provides a good predictive model to estimate the thermal conductivity of vegetable and fruit tissues at different dehydration stages. The selected products are quite different in structure and composition and behave differently during dehydration in their shrinking pattern. Nonetheless the theory gives reasonably good results provided an expression for the porosity, of the type $\epsilon = \epsilon(X)$, is available.

It must be noticed that experimental data of k_m (X) and ϵ (X) for apples have been taken on different samples and at different times. The fact that EMT provides a good estimation can be taken as a crosscheck of those results.

Finally, Eq. (11) and (12) are general correlations for potatoes and pears thermal conductivities as a function of moisture content, which represent useful information in drying analysis.

NOMENCLATURE

k	Thermal conductivity (W/m°C)
v	Phase volume
X	Water content (g water/g dry matter)
Z	Coordination number

Greek

ϵ	Volume fraction, also porosity
δ	Density (gm/cm^3)
σ	Sugar/dry matter ratio

Subscripts

a	air
c	cell, also continuous
d	disperse
g	starch granules
i	i-th phase
m	average
o	initial
s	starch
w	water

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Thawing Time of Frozen Food of a Rectangular or Finitely Cylindrical Shape

CARLO NONINO and KAN-ICHI HAYAKAWA

ABSTRACT

Through dual step simulations, theoretical regression formulae were obtained for estimating the dimensionless thawing time of rectangular or cylindrical food. These formulae contain independent variables related to an initial uniform food temperature, final central food temperature, thawing medium temperature, highest freezing point of food, empirical constant for estimating food enthalpy, shape factor of food and Biot number. The formulae were verified for their reliability by comparing thawing times estimated by these formulae with those determined experimentally. Error analyses were performed by using the formulae to examine the errors of thawing times due to uncertainty in parametric values.

INTRODUCTION

SINCE THE QUALITY of frozen food is greatly influenced with either thawing or freezing, many researchers have developed means for predicting the freezing or thawing time of food. Since there was no method available for predicting the freezing time of finitely cylindrical or rectangular food by manual calculations, such a procedure has been developed in previous investigation (Hayakawa et al., 1983b). De Michelis and Calvelo (1983) published their procedure to estimate the freezing time of finitely cylindrical or rectangular parallelepiped food by modifying Plank's (1941) equation.

A method may be used interchangeably to estimate a freezing or thawing time if it is developed by assuming that freezing is completed at a single temperature and that property values are independent of temperature and are constant within a frozen or unfrozen phase. Since these assumptions were unrealistic, more realistic conditions were used to develop our previous method. Furthermore, it is important to note that there was no published method available for estimating the thawing time of a rectangular or finitely cylindrical body by manual or simple computation. Therefore, the present work was initiated.

Some physical operational and dimensional parameters have been known to influence a thawing rate of frozen food. Several researchers (Hayakawa et al., 1983b; Hsieh et al., 1977; Succar and Hayakawa, 1983) examined this influence on the freezing rate of selected food. However, there is no published information available on the significance of all independent parameters on the thawing rate. Therefore, one of our objectives was to identify significant parameters for thawing the food of the assumed shapes.

PARAMETRIC ANALYSIS

A GENERAL APPROACH for accomplishing our objectives was similar to previous work (Hayakawa et al., 1983b). A mathematical model was used to develop theoretical regression formulae for estimating the thawing time. This model simulated transient state, anisotropic heat conduction equation and convective radiative and moisture loss boundary conditions. This model was identical to the one described in a previous paper (Hayakawa et al., 1983). There are several typographical errors in the equations given in the paper: 1. Delete $(T_{sw} - T_{sh})$ from Eq. (9); 2. Replace C_e in Eq. (16) with C_r; 3. Replace $(T_{sw} - T_{sh})$ in Eq. (16) by $1/(n_c - 1)$; 4. Replace $(T_{sw} - 1)$ in Eq. (16) by $(T_{sw} - T)$; 5. Replace $n_c - 1$ in Eq. (27) with n_c). A heat conduction equation was used to model heat transfer in a sample body whose thermal conductivities for directions parallel to coordinates axes were different from each other. In this equation, the latent heat of phase change was included in temperature dependent apparent heat capacity. Thus, a mathematically difficult moving boundary problem was reduced to a regular heat conduction problem. All temperature dependent thermophysical properties of a sample were assumed to be location-independent and were estimated by Schwartzberg's or modified Schwartzberg's formula (Succar and Hayakawa, 1983).

For the boundary conditions of the heat conduction equation, the coefficients of surface heat transfer, coefficient of surface moisture transfer and also surface adsorptivity of radiative heat were assumed to be location-variable and time-invariable. It was further assumed that moisture loss or gain was proportional to a vapor pressure difference between the bulk air and air adjacent to the sample surface. The vapor in the air adjacent to the surface was assumed to be saturated at the surface temperature. Because of a temperature difference between the sample surface and bulk air, the vapor pressure difference was estimated after converting them to pressures at a common temperature by using an ideal gas law. The vaporization, sublimation or condensation of water was assumed to take place on the surface. Finally, the latent heat for each of the named phase conversions was assumed to be a linear function of temperature.

The following are the independent dimensionless parameters in the model which are classified according to their physical characteristics.

Thermal conductivities

$$M_{klx} = S_{klx}(T_{or} - T_{ar})/k_{rx}$$
$$M_{ksx} = S_{ksx}(T_{or} - T_{ar})/k_{rx}$$
$$\gamma = k_{ry}/k_{rx}$$
$$R_{kx} = k_{lx}/k_{rx} - 1$$
$$M_{kly} = S_{kly}(T_{or} - T_{ar})/k_{rx}$$

Density

$$R_d = \rho_l/\rho_r - 1$$
$$M_d = S_d(T_{or} - T_{ar})/\rho_r$$

Apparent specific heat

$$R_c\ C_l/C_r$$
$$C_{eh} = E/[C_r(CT_{sw} - T_{sh})^{n_c}]$$
n_c (dimensionless constant)

Surface heat or mass transfer coefficient

$$Bi_u = h_{qu}\ell/k_{rx}$$
$$Bi_n = h_{qn}\ell/k_{lx}$$
$$Bm_u = h_{mu}L_rP_r\ell/[(T_{or} - T_{ar})k_{rx}]$$
$$Bm_n = h_{mn}L_rP_r\ell/[(T_{or} - T_{ar})k_{rx}]$$
$$NR_u = \sigma F\epsilon_u\ell(T_{or} - T_{ar})^3/k_{rx}$$
$$NR_n = \sigma F\epsilon_n\ell(T_{or} - T_{ar})^3/k_{rx}$$

Operational constant

$$U_o = (T_o - T_{ar})/(T_{or} - T_{ar})$$
$$U_{sh} = (T_{sh} - T_{ar})/(T_{or} - T_{ar})$$
$$U_p = (T_p - T_{ar})/(T_{or} - T_{ar})$$
$$U_a = (T_a - T_{ar})/(T_{or} - T_{ar})$$
$$U_f = (T_f - T_{ar})/(T_{or} - T_{ar})$$
$$SF = \ell_v/\ell$$

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Table 1—Maximum, minimum, and frequently observed values of dimensionless parameter[a]

Parameter	Minimum	Frequent	Maximum
		Thermal conductivity	
M_{klx}	−0.4634	−3.52(−2)	−2.8(−3)
R_{kx}	−0.7595	−0.7080	−0.6301
M_{ksx}	−0.5845	−0.07	0.1275
M_{kly}	−0.6367	−5.58(−2)	−3.83(−3)
γ	0.866	0.917	0.958
		Density	
R_d	1.7(−2)	5.6(−2)	8.1(−2)
M_d	−1.1(−2)	3.65(−3)	3.6(−2)
		Apparent specific heat	
R_c	1.83	2.71	5.40
C_{eh}	19.4	1.57(2)	7.69(2)
n_c	1.41	1.78	2.00
U_{sh}(C_{eh} dependent)	0.171	0.209	0.313
		Surface heat or mass transfer coefficient	
Bi_u(U_a > −1.917)	6.35(−3)[8.47(−3)]	0.297[0.416]	35.30(22.99]
Bi_u(U_a < −1.917)	6.35(−3)[8.47(−3)]	0.105[0.147]	1.412[0.920]
Bi_n		Dependent on Bi_u	
Bm_u(U_a > −0.5625)	−2.30(−3)[−1.50(−3)]	−2.08(−5)[−2.77(−5)]	0.0
Bm_u(U_a < −0.5625)	0	0	0
Bm_n		$Bm_n > Bm_u$	
NR_u	−9.42(−4)[−6.14(−4)]	−8.55(−6)[1.14(−5)]	0
NR_n		$NR_n > NR_u$	
		Operational conditions	
U_o	0.292	0.58	0.79
U_a(U_f dependent)	−8.16	−0.229	0.146
U_f(U_{sh} dependent)	−1.50	6.25(−2)	0.291
U_p	U_a − 5/48	U_a	U_a + 1/48
ϕ_a	0.2	0.7	1.0
Sf	0.5[0.125]	4[2.4]	16[5]
ℓ(cm)[b]	0.3[0.4]	2.5[3.5]	15.35[10.0]

[a] A signed integer placed within a pair of parentheses is a power of 10 which should be multiplied to obtain a correct value. All listed values except those given two values in one column are applicable both to cylindrical and to rectangular food. When two values are given, the first is for rectangular food and the bracketed, second value, for cylindrical food. For example, 0.5 is the minimum shape factor, Sf, for rectangular food and 0.125 is for cylindrical food.

[b] This is a characteristic dimension, which is dimensional quality.

Table 2—Limiting values of interrelated parameters[a]

Parameter A	Level of A	Value of A	Parameter B	Minimum of B	Maximum of B
C_{eh}	min	19.40	U_{sh}	0.171	0.313
	max	769.0		0.171	0.190
U_{sh}	min	0.171	U_f	−1.500	0.150
	max	0.191		−1.500	0.292
U_f	min	−1.500	U_a	−8.160	−1.604
	max	0.292		−0.563	0.146
Bi_n (rec)	min	0.0064	Bi_u (rec)	0.0064	0.0064
	max	35.2		0.0064	35.2
Bi_n (cyl)	min	0.0084	Bi_u (cyl)	0.0084	0.0084
	max	23.0		0.0084	23.0
Bm_u (rec)	min	−0.0023	Bm_n (rec)	−0.0023	0.0
	max	0.0		0.0	0.0
Bm_u (cyl)	min	−0.0015	Bm_n (cyl)	−0.0015	0.0
	max	0.0		0.0	0.0
NR_u (rec)	min	−9.42(−4)	NR_u (rec)	−9.42(−4)	0.0
	max	0.0		0.0	0.0
NR_u(cyl)	min	−6.14(−4)	NR_u(cyl)	−6.14(−4)	0.0
	max	0.0		0.0	0.0

[a] cyl = cylindrical food; rec = rectangular food.

Table 3—Analysis of variance on screening simulation

Parameters	Rectangular food		Cylindrical food	
	F value	Level of significance	F value	Level of significance
U_o	2.60	0.1200	2.32	0.1409
U_a	24.72	0.0001*	16.80	0.0004*
U_{sh}	6.14	0.0206*	3.64	0.0684**
U_f	0.64	0.4319	0.41	0.5271
U_p	0.16	0.6951	0.31	0.5821
R_{kx}	0.77	0.3891	0.02	0.8842
M_{ksx}	<0.005	0.9905	0.10	0.7570
M_{klx}	0.09	0.7678	0.45	0.5068
M_{kly}	0.07	0.7874	0.14	0.7107
γ	0.18	0.6751	0.26	0.6130
R_d	0.12	0.7324	0.11	0.7425
M_d	<0.005	0.9879	0.09	0.7633
R_c	1.02	0.3227	0.39	0.5363
C_{eh}	9.37	0.0054*	8.95	0.0063*
n_c	0.69	0.4155	0.01	0.9146
Bi_u	84.94	0.0001*	58.73	0.0001*
Bi_n	3.69	0.0667**	2.26	0.1456
Bm_u	1.88	0.1836	1.85	0.1866
Bm_n	1.20	0.2832	0.15	0.7034
NR_u	0.05	0.8286	0.01	0.9247
NR_n	0.01	0.9217	0.15	0.7065
ϕ_a	0.18	0.6746	1.18	0.2875
Sf	7.21	0.0130*	75.96	0.0001*

* Significant influence on defrosting time

** Nearly significant influence on defrosting time

Explicit expressions for estimating temperature dependent physical property values are given in a previous paper (Hayakawa et al., 1983a).

To determine the values of the above parameters, one may assume any reasonable temperatures as the reference initial food temperature, T_{or}, and reference surrounding medium temperature, T_{ar}. For our previous work, we assumed that $T_{ar} = -40$°C and $T_{or} = 8$°C. For the present work, these temperatures were interchanged, i.e., $T_{ar} = 8$°C and $T_{or} = -40$°C. Because of this interchange, the signs of the parameters (M_{klx}, M_{ksx}, M_{kly}, M_d, Bm_u, Bm_n, NR_u, NR_n) were reversed although their absolute values were identical to those given in the previous paper (Hayakawa et al., 1983b).

In addition, the dimensionless, operational temperatures were changed since they were different for thawing processes. A radiation source temperature is likely close to a surrounding heating medium temperature in most cases. Therefore, to calculate U_p, this temperature was assumed to be between $T_a - 1$ and $T_a + 5$ (°C). Surrounding, convective heat exchange medium temperatures were assumed to be between 1 and 400°C. A fairly high upper limit was assumed since an effective medium temperature for thawing in an oven could be as high as this limit. The initial food temperature was assumed to be uniform and between −30°C and −6°C. The minimum and maximum food temperatures at its thermal center were $T_{sh} + 1$ and 80°C, respectively, when thawing was completed.

According to the survey of literature listed in the previous paper (Hayakawa et al., 1983b), surface heat conductance, h_q, was likely between 0.4 and 20 [cal/(hr cm^{2}C°)] when a heating medium temper-

Table 4—Minimum, frequent, and maximum values used for fractional, central composite simulation

Parameter	Minimum	Frequent	Maximum
$-U_o$	−0.79	−0.58	−0.29
C_{eh}	19.4	157.14	769.0
$-U_{sh}$	$0.0766501 \log_{10}C_{eh} - 0.41121$	$0.0406288 \log_{10}C_{eh} - 0.2983218$	−0.171
$-U_f$	$0.999293(-U_{sh}) + 0.0206792$	$0.925088(-U_{sh}) + 0.130990$	1.500
$-U_a$	$0.976742(-U_f) + 0.138988$	$1.338189(-U_f) + 0.322716$	$4.24062(-U_f) + 1.79907$
		Rectangular Food	
$1/Bi_u$		when $U_a \geq -1.91666$ (Ta≤100°C)	
	0.02833	3.365	157.5
$1/Bi_u$		when $U_a < -1.91666$ (Ta>100°C)	
	0.708	9.53	157.5
$1/Bi_n$		when $U_a \geq -1.91666$ and $0.02833 \leq 1/Bi_u \leq 3.365$	
	$0.077222/Bi_u + 0.0261469$	$0.908602/Bi_u + 0.307555$	$3.86134/Bi_u + 1.30659$
$1/Bi_n$		when $U_a > -1.91666$ and $3.365 < 1/Bi_u \leq 157.5$	
	$0.0185811/Bi_u + 0.223475$	$0.218607/Bi_u + 2.62939$	$0.929056/Bi_u + 11.1737$
$1/Bi_n$		when $U_a \leq -1.92666$ and $0.708 \leq 1/Bi_u \leq 9.533$	
	$0.729972/Bi_u + 0.19118$	$0.973201/Bi_u + 0.255474$	$1.45994/Bi_u + 0.38236$
$1/Bi_n$		when $U_a < -1.91666$ and $9.533 < 1/Bi_u \leq 157.5$	
	$0.483892/Bi_u + 2.53706$	$0.645191/Bi_u + 3.38239$	$0.967783/Bi_u + 5.07412$
Sf	0.5	4.0	16.0
		Cylindrical Food	
$1/Bi_u$		when $U_a \geq -1.91666$ (Ta≤100°C)	
	0.0435	2.403	118.13
$1/Bi_u$		when $U_a < -1.91666$ (Ta>100°C)	
	1.0875	6.808	118.13
$1/Bi_n$		when $U_a \geq -1.91666$ and $0.0435 \leq 1/Bi_n \leq 2.403$	
	$0.0681076/Bi_u + 0.0405373$	$0.801568/Bi_u + 0.476832$	$3.40538/Bi_u + 2.02687$
$1/Bi_n$		when $U_a \geq -1.91666$ and $2.403 < 1/Bi_n \leq 118.125$	
	$0.0186507/Bi_u + 0.159382$	$0.219379/Bi_u + 1.87583$	$0.932537/Bi_u + 7.96911$
$1/Bi_n$		when $U_a < -1.91666$ and $1.0875 \leq 1/Bi_u \leq 6.808$	
	$0.702509/Bi_u + 0.323522$	$0.936631/Bi_u + 0.431413$	$1.40460/Bi_u + 0.647500$
$1/Bi_n$		when $U_a < -1.91666$ and $6.808 < 1/Bi_u \leq 157.5$	
	$0.484704/Bi_u + 1.80634$	$0.646280/Bi_u + 2.40812$	$0.969439/Bi_u + 3.61006$
Sf	0.125	2.4	5.0

Table 5—Values of C_{eh} and U_{sh} of selected samples

Product	Moisture[a]	C_{eh}	U_{sh}[d]
Tylose MH 1000	0.77	206.7	0.1791
Tylose MH 1000 + NaCl	0.77	157.91	0.1865
Beef meat (lean)	0.74	114.20	0.1873
	0.70	107.90	0.1877
	0.63	58.50	0.2033
	0.57	57.33	0.2088
	0.45	39.52	0.2519
Fish meat (lean)	0.82	171.91	0.1833
	0.75	147.52	0.1875
	0.66	59.15	0.2073
	0.57	32.46	0.2283
Fruit & Vegetable Juices[c]	0.96	517.00	0.1748
	0.87	114.48	0.1954
	0.75	33.07	0.2331
	0.61	19.44	0.3121
Sucrose solution	0.96	769.59	0.1710
	0.61	68.38	0.2575

[a] Mass fraction of water in sample

[b] Properties of this cellulose gel are similar to those of lean beef meat with moisture fraction of 0.77

[c] Also applicable to fresh fruit or vegetables

[d] Because the present values of T_{ar} and T_{or} are different from those used for estimation of freezing time (Hayakawa et al., 1983a), these U_{sh} cannot be used for freezing time estimation.

ature, T_a, was less than 100°C since water or air was frequently used as a heating medium. Furthermore, the range of h_q values was narrower between 0.4 and 0.8, when $T_a > 100$ since hot air was frequently used. These h_qs were used to calculate Bi values.

The relative humidity of hot air for thawing is very low. Therefore, its lower limit was assumed to be 0.2. The upper limit was assumed to be 1.0 for cases with negligible moisture loss. When a thawing temperature was higher than 100°C, food was likely covered tightly for most cases. Therefore, Bm was assumed to be zero when U_a was beyond this limiting level. Table 1 shows the maximum, minimum and frequently observed values of each parameter estimated from the data collected by our literature survey.

As observed previously (Hayakawa et al., 1983b), the limits of several parameters were dependent on other parameters although any values could be assigned independently within these limits. Table 2 summarizes the limiting values of parameters (B) as influenced with the limiting values of other parameters (A). In addition to those listed, Bm and NR were influenced by Bi_u since the ratio, l/k_{rx}, is included in their respective parameters. However their maximum ranges (Table 1) were used for our preliminary analysis to obtain complete information on these parameters.

Two stage computerized simulations were performed to investigate the thawing rate. The first was screening simulation to identify dimensionless parameters which influenced significantly the thawing rate and the second was regression simulation to determine functional relationship between the thawing time and significant parameters. Since the general approach for both simulations was similar to those described previously, the details of this approach are not repeated in the present paper.

As for the regression simulation, one quarter replicate of a central composite design of experiments was selected to generate theoretical thawing times for statistically sound combinations of significant parameters selected by the screening simulation. In the selected design, the minimum and maximum levels of each design variable, X_j, were set to −2.82843 and 2.82843, respectively, and the three additional values, −1.0, 0.0, 1.0 were assigned to it for the simulation. It should be stated that the minimum, frequent, and maximum values of each parameter should be transformed to −2.82843, 0.0, and +2.82843 of the corresponding design variable respective.

Since the limiting values of each parameter were not equal to those of the design variable, the following equations were used to estimate parametric values, P_j, which corresponded to respective levels of a design variable, X_j,

$$X_j = D_{j1} \ln(P_j + D_{j2}) + D_{j3} \quad (1)$$

where constants D_{j1}, D_{j2} and D_{j3} were estimated from the minimum, frequent, and maximum values of P_j by:

$$D_{j1} = 2s/\ln [(P_{j,max} + D_{j2})/(P_{j,min} + D_{j2})] \quad (2)$$

$$D_{j2} = (P_{j,min} \cdot P_{j,max} - P_{j2,\,frq})/(2P_{j,\,frq} - P_{j,max} - P_{j,min}) \quad (3)$$

$$D_{j3} = -D_{j1} \ln(P_{j,\,frq} + D_{j2}) \quad (4)$$

Where s = 2.82843 for the present simulation.

Table 6—Comparison of experimental and theoretical thawing times of samples

Sample[a] (Moisture %)	Sample no.	Sample[b] size (cm × cm)	h_q[c] cal/(hr cm²C°)	T_o (°C)	T_a (°C)	Thawing time (hr) for the following[d] T_f°C					
						5		10		15	
						Expt	Theo	Expt	Theo	Expt	Theo
Lean beef (73.5)	1	5.75 × 8.10	1.77(0.698)	−10	23.4	2.96	3.11(5.1)	3.30	3.34(1.2)	4.08	3.64(−10.8)
	2	"	"	"	"	2.89	3.11(7.6)	3.32	3.34(0.6)	4.08	3.64(−10.8)
	3	"	"	"	"	2.99	3.11(4.0)	3.30	3.34(1.2)	4.08	3.64(−10.8)
Cellulose gel (77.9)	1	3.30 × 7.60	1.32(0.322)	−12.7	22.7	2.47	2.43(−1.6)	2.53	2.55(−1.2)	2.86	2.85(−0.3)
	2	"	"	"	"	2.46	2.43(−1.6)	2.56	2.55(−0.4)	2.86	2.85(−0.3)
	3	"	"	"	"	2.38	2.43(2.1)	2.49	2.55(2.4)	2.78	2.85(2.5)
	4	3.30 × 12.40	1.17(0.285)	−12.7	22.7	2.63	2.66(1.1)	2.78	2.79(0.3)	3.08	3.13(1.6)
	5	"	"	"	"	2.69	2.66(−1.1)	2.85	2.79(−2.1)	3.17	3.13(−1.2)
	6	"	"	"	"	2.66	2.66(−0.7)	2.79	2.79(−0.7)	3.13	3.13(0.0)

[a] The value in parenthesis is the average moisture content (%) of each sample.

[b] For the lean beef meat, the first and second values are the rectangular side dimensions perpendicular to and parallel to the meat fibers respectively. Difference in the dimensions of individual samples are 1 mm or less. For the cellulose gel, they represent the radius and height of a cylindrical sample. For both samples, the first values are their characteristic dimensions (ℓ)

[c] The value in the parenthesis is Biot number.

[d] Expt = experimentally determined times, theo = times estimated theoretically by using the regression formulae. The value in a pair of parenthesis placed by each theoretically estimated thawing time is its error (%).

Table 7—Values of significant parameters and of corresponding design variables for test samples

Parameters	Lean beef meat		Cellulose gel[a]	
	Parametric value	x_i	Parametric value	x_i
$-U_o$	−0.3750	2.090	−0.4208	1.664
$-U_a$[b]	0.3217	0.5031 −0.3908 −1.760	0.3063	0.4223 −0.5197 −2.078
$-U_{sh}$	−0.1873	1.812	−0.1791	2.580
U_f	−0.06250	−0.1821	Identical to lean beef meat	
	0.04167	0.5427	Identical to lean beef meat	
	0.1458	0.9795	Identical to lean beef meat	
C_{eh}	114.2	−0.5245	206.7	0.5544
$1/Bi_u$[c]	1.437	0.6149	3.106 3.509	0.1861 0.2746
$1/Bi_n$[c]	1.437	−0.2001	3.106 3.509	0.3476 0.5091
Sf[c]	1.41	−1.705	2.303 3.758	−0.1132 1.524
α_r	42.05 cm²hr	−	42.98 cm²/hr	−

[a] Values of Karlsruhe test substance

[b] The three values of the design variable, X_a, correspond to the three U_f

[c] The first and second values of the parameters and corresponding design variables for the cellulose gel are for the smaller and larger can sizes respectively.

MATERIALS & METHODS

TO VERIFY the validity of theoretical regression formulas, rectangular and cylindrical samples were prepared from lean beef meat and cellulose gel as described in a previous paper (Hayakawa et al., 1983a). Five rectangular samples were made by trimming the meat. The size of each sample was 8.13 × 5.75 × 3.00 cm³. The 8.13 cm side was parallel to the direction of the meat fibers. To ensure two dimensional heat conduction in each sample, the two opposing 8.13 × 5.75 cm² surfaces were covered with 3 cm thick expanded polystyrene slabs.

To prepare the cylindrical samples, 77% cellulose gel, known as Karlsruhe test substance, was filled in a cylindrical body made from the side of 211 × 300 or 211 × 414 can. The open top and open bottom ends of the cylinder were covered with polyethylene film, 0.00254 cm thick, to provide support for the gel and to minimize the evaporative loss of moisture from the gel. Five samples were prepared for each cylindrical size. One copper-constantan thermocouple junction was placed at the geometrical center of each sample to monitor sample temperature during a thawing experiment. The location of thermocouple in each meat sample was verified by taking X-ray pictures after completing the experiments.

The prepared samples were suspended with fine strings from a frame made of aluminum rods and frozen in a −10°C or −12.2°C forced air high-low temperature chamber (Associated Testing Laboratories Inc., Model SK-3105). A uniform temperature distribution in each sample was ensured by monitoring its central temperature before each thawing experiment. The experiment was started by exposing the samples to a room temperature, about 23°C. During the experiment turbulant air flow around the samples was created by two 30 cm diameter fans facing each other and centering the samples. The temperature history of each sample was recorded by a multipoint strip chart recorder (Honeywell Electronik 16). There were virtually no changes in the room temperatures during the experiments.

RESULTS & DISCUSSION

TABLE 3 shows abridged results obtained through the statistical analysis of variations of the results obtained through the screening simulation. With rectangular food, the significant parameters are the convective heating medium temperature (U_a), final thawing or initial freezing point (U_{sh}), a constant for estimating apparent specific heat (C_{eh}), Biot number applicable to the three sides of food, (Bi_u), and shape factor (Sf). In addition, Biot number applicable to the remaining side (Bi_m) was nearly significant. With cylindrical food, U_a, C_{eh}, Bi_u, and Sf were significant and U_{sh} was nearly significant.

It is of practical interest to note that the thawing rates of both foods were not influenced significantly by the initial and final food temperatures, temperature dependency and anisotropy of thermal conductivity, temperature dependency of density, radiative heat transfer, and moisture loss. For our further analysis, the following eight dimensionless parameters were examined: U_o, U_a, U_{sh}, U_f, C_{eh}, Bi_u, Bi_n, and Sf. Among the eight parameters, two nonsignificant parameters, U_o and U_f, were included since they were operationally important. In addition, Bi_n was included for the cylindrical food for the same reason.

Table 4 shows the minimum, frequent and maximum values of each parameter used for the simulation. Four parameters, U_o, U_{sh}, U_f and U_a, were multiplied with −1.0 to apply Eq (1) through (4) since these equations could be used only when $P_{j,freq}$ was less than the median of $P_{j,min}$ and $P_{j,max}$. Through-

Table 8—Errors in thawing times owing to errors in all significant parameters

Source of Error	Rectangular food		Cylindrical food	
	Error (hr)	%[a]	Error (hr)	%[a]
Operational temp	0.3418	10.20	0.4910	8.54
Physical property				
E	0.0605	1.80	0.1035	1.80
C_r	0.1478	4.41	0.2135	3.71
T_{sh}	0.0185	0.55	0.0370	0.64
k_r	0.0737	2.20	0.1743	3.03
ρ_r	0.1677	5.00	0.2875	5.00
Sub total[b]	0.4682	13.96	0.8158	14.19
Surface coefficient	0.1867	5.57	0.2262	3.93
Food dimension	0.1817	5.41	0.2265	3.94
Total	1.1783	35.15	1.7595	30.60

[a] The percent error of thawing time

[b] The sum of errors owing to all significant property values.

out the central composite simulation, parameters not listed in Table 4 were kept at their frequent levels.

Eighty-one dimensionless thawing times were obtained for each food when the above stated design was applied for the simulation. These times were then subject to regression analyses by using a computer program package available at our computer center. After a number of trial analyses, regression formulae with very high r^2 values for both configurations were obtained. These formulae are given below together with their r^2 values.

Rectangular food

$$\ln(Fo_t) = 3.2127564 - 0.0191845X_o - 0.2773152X_a - 0.1918885X_{uh} - 0.2625484X_f + 0.3339468X_{eh} + 0.7469373X_{iu} + 0.1232592X_{in} + 0.0590265X_{sf} - 0.0083424X_aX_{uh} - 0.0231854X_aX_{iu} + 0.0159873X_{uh}X_f - 0.0152391X_{uh}X_{eh} - 0.0287775X_fX_{eh} - 0.0224377X_fX_{iu} + 0.0593209X_{iu}X_{in} + 0.0191681X_{iu}X_{sf} + 0.0161366X_{in}X_{sf} - 0.0380997X_o^2 - 0.0746406X_a^2 - 0.0891996X_{uh}^2 - 0.0369378X_f^2 - 0.0598422X_{eh}^2 + 0.063829X_{iu}^2 - 0.0403200X_{in}^2 - 0.0811637X_{sf}^2 - 0.0039272X_o^3 - 0.0164493X_{uh}^2 + 0.0127131X_f^3 - 0.0056566X_{iu}^3 + 0.0049656X_{in}^3 + 0.0169607X_{sf}^3 \quad (5)$$

$$r^2 = 0.989$$

Finitely cylindrical food

$$\ln(Fo_t) = 2.9827634 - 0.0138364X_o - 0.2465853X_a - 0.1854159X_{uh} - 0.2589734X_f + 0.3383583X_{eh} + 0.5488136X_{iu} + 0.0337506X_{in} + 0.1369748X_{sf} - 0.0117502X_o^2 - 0.0421749X_a^2 - 0.0098833X_aX_{eh} - 0.0256559X_aX_{iu} - 0.06170606X_{uh}^2 + 0.0136849X_{uh}X_f - 0.0152869X_{uh}X_{eh} - 0.0209326X_f^2 - 0.0259682X_fX_{eh} - 0.0232858X_fX_{iu} - 0.0355150X_{eh}^2 + 0.1315991X_{iu}^2 + 0.01715360X_{iu}X_{in} - 0.0192510X_{in}X_{sf} - 0.1283057X_{sf}^3 - 0.0037055X_o^3 - 0.0156848X_{uh}^3 + 0.0104680X_f^3 + 0.0022558X_{eh}^3 + 0.0091084X_{iu}^3 + 0.03675536X_{sf}^3 \quad (6)$$

$$r^2 = 0.999$$

For the equation of rectangular food, the maximum difference between the predicted and computer estimated thawing times was less than 8% when the values of each design variable was within the closed range between −1.0 and +1.0 and less than 0.01% when they were all equal to zero. When the values of the variables were outside of the closed ranges, the maximum difference was about 20%. For the equation of a finitely cylindrical food, the differences were distributed uniformly throughout the space of the design variable, the maximum difference being less than 5% within the closed ranges of the design variables, between −1.0s and +1.0s and less than 4.5% outside of the ranges. The difference at the zero point was 0.8%.

To use Eq. (5) and (6), two food property values, C_{eh} and U_{sh} are required. Table 5 shows these values calculated from published data (Succar and Hayakawa, 1983).

Temperature history curves for the five meat samples and curves for the five cellulose gel samples of each can size were obtained. Two out of these five curves were used to determine the values of the convective surface heat transfer coefficient, h_q values. It should be noted that all other physical property values of lean beef meat and of cellulose gel, Karlsruhe test substance, are known (Hayakawa et al., 1983a). Therefore, the h_q value is only one unknown parametric value required for predicting a thawing time. The remaining three temperature history curves were used to determine experimental thawing times (Table 6).

As our preliminary estimation, the h values for the meat samples and for the cellulose gel of the smaller can size were determined. This determination was accomplished by minimizing the sum of squares of differences in sample temperatures on experimentally determined history curves and those on respective history curves estimated theoretically. The latter curves were estimated by using our computer program developed previously. Another set of h_q values was determined by used Eq. (5) and (6) together with experimentally determined thawing times required for central sample temperatures to reach 5, 10 and 15 (°C). As in our previous work (Hayakawa et al., 1983b), the h_q values determined by both procedures were virtually identical. Therefore, those determined by applying Eq. (5) and (6) were used to estimate the thawing times of the three remaining samples of each group. To use the equations, the values of eight design variables were required. Therefore, these values were estimated by using the parametric values given in Table 7, their minimum, frequent and maximum values given in Table 1 and Eq. (1) through (4). The estimated values are given in Table 7. Table 6 shows the thawing times determined experimentally and theoretically. The differences between the theoretical and experimental thawing times were less than 10% in most cases and the maximum difference was about 11%. There was better agreement in the thawing times for the cylindrical samples.

Although there was reasonably good agreement between the experimental and theoretical thawing times of rectangular samples, their differences were greater than those for the cylindrical samples. These greater differences were likely caused by the comparatively goodness of fit of the equation for rectangular food, Eq. (5), and by probably greater deviations in the U_{sh}, C_{eh} and α_r values of sample beef meat from the respective literature values owing to difficulty in controlling its compositions compared with the cellulose gel, and by difficulty in completely insulating the two opposing surfaces of each rectangular sample, and by difficulty in forming the rectangular shape out of the meat.

The samples used for the experiments were covered by polyethylene film and/or tin-plate. Therefore, h_q values listed in Table 5 are overall heat transfer coefficients comprising the convective conductance of the outside surface, conduction conductance of film or plate and contact conductance of inside surface. The thermal conductivities of polyethylene and of steel are about 2.6 and 16.0 cal/(hr cm C°) respectively. The thickness of polyethylene film and tin-plate of cans used for the experiments are about 0.0025 and 0.038 cm respectively. Therefore, the resistances of the film and plate are 0.00097 and 0.00028 hr $cm^2C°$/cal respectively. According to the h_q values given in Table 6, the overall resistances, $1/h_q$, are from 0.56 to 0.85 hr $cm^2C°$/cal, which are over 500 times greater than those of the film and plate. Therefore, the h_q values determined comprise virtually the outside and inside conductances.

It is practically useful to find the influence of all parametric errors on the thawing time. Therefore, an error analysis was performed using Eq. (5) and (6). To simplify these analyses, all parametric values were assumed to be at the frequently observed levels. Since an overall mathematical technique for the error analysis was similar to the one for previous work (Hayakawa et al., 1983b), the final equations are given below.

Rectangular food

$$|\Delta t| < 0.01543|\Delta T_o| + 0.04067|\Delta T_f| + 0.1428|\Delta T_a| + 0.01511|\Delta E| + 7.393|\Delta C_r| + 0.1846|\Delta T_{sh}| + 0.1024|\Delta k_{rx}| + 3.353|\Delta \rho_r| + 0.6855|\Delta h_u| + 0.4259|\Delta h_n| + 0.03677|\Delta W| + 1.779|\Delta \ell| \quad (7)$$

Cylindrical food

$$|\Delta t| < 0.01908|\Delta T_o| + 0.03695|\Delta T_f| + 0.2179|\Delta T_a| + 0.02588|\Delta E| + 10.675|\Delta C_r| + 0.3708|\Delta T_{sh}| + 0.2422|\Delta k_{rx}| + 5.751|\Delta \rho_r| + 1.298|\Delta h_u| + 0.04839|\Delta h_n| + 0.2619|\Delta W| + 2.004|\Delta \ell| \quad (8)$$

It should be noted that the error of reference density, ρ_r, was included in Eq. (7) and (8) although the dimensionless thawing time was not significantly influenced by the food density. This apparent contribution was due to the fact that the reference density was included in the dimensionless thawing time. The following reasonable errors were assumed for all significant parametric values to use above Eq. (7) and (8).

Operational temperatures

$$\Delta T_o = \Delta T_f = 1\ C^\circ \qquad \Delta T_a = 2\ C^\circ$$

Physical properties of food

$$\Delta T_{sh} = 0.1\ C^\circ \quad \Delta E = 4\ cal/g(C^{\circ 1-n_c})$$

$$\Delta C_r = 0.02\ cal/(gC^\circ)$$
$$\Delta k_{rx} = 0.720\ cal/(hr\ cm\ C^\circ)$$
$$\Delta p_r = 0.05\ g/cm^3$$

Surface heat transfer coefficient

$$\Delta h_u = \Delta h_n = 0.168\ cal/(hr\ cm^2\ C^\circ)$$

Food dimension

$$\Delta \ell_v = \Delta \ell = 0.1$$

Among the above listed, the errors in all physical property values except ΔT_{sh} were 5% of their frequently observed values, and the errors of the surface heat transfer coefficients were 10% of their frequent values. These errors yielded errors in the thawing time shown in Table 7. The same table also shows the percent errors of the thawing times at frequent parametric values. According to Eq. (5) and (6), the thawing times of the rectangular and cylindrical foods were 3.353 and 5.751 hr, respectively. It should be noted that the longer thawing time of the cylindrical food was due to its larger size, 3.5 cm radius and 8.4 cm height, compared to the rectangular food, 2.5 cm wide and 10 cm long side dimensions.

The errors in the operational temperatures and in the physical property values (Table 7) caused about 10 and 14% errors in the thawing times, respectively, the error in the surface heat transfer coefficients, h_q, or in the food dimension caused about 5% error. Among the physical properties, the reference density, ρ_r, contributed most to the error of the thawing time, 5%. The reference specific heat, C_r, and reference thermal conductivity, k_r, contributed about 4 and 3% errors, respectively. To reduce an error in the thawing time, one should accurately determine the values of h_q, ρ_r, C_r and k_{rx}. Since ρ_r and C_r are relatively easier to determine than h_q and k_r, the reliability of an estimated thawing time may be improved economically by accurately determining ρ_r and C_r. Hsieh et al. (1977) observed similarly.

An h_q value is considerably difficult to determine since a reliable mathematical model for simulating surface heat transfer is required. Equations (5) and (6) may be used to determine this h_q value easily when one has an experimentally determined thawing time, provided that the physical property values of a rectangular or cylindrical sample are known and that all parametric values are within the minimum and maximum values shown in Table 1.

NOMENCLATURE

Bi	$= h_q \ell / k_{rx}$. Dimensionless convective surface heat transfer coefficient.
$B_{m'}$	$= h_m L_r \rho_r \ell / [(T_{or} - T_{ar}) k_{rx}]$. Dimensionless convective surface mass transfer coefficient.
C_{eh}	$= E/[C_r(T_{sw} - T_{sh})^{nc}]$. Empirical constant for estimating dimensionless apparent specific heat of food at temperatures below U_{sh}.
C_l	Specific heat of unfrozen food [cal/(g C°)].
C_r	Reference specific heat of food. This is a constant in an empirical formula for estimating apparent specific heat of food temperatures below T_{sh}. [cal/(g C°)].
D_{j1}, D_{j2}, D_{j3}	Constants required for transforming significant dimensionless parameters to corresponding design variables.
E	Constant in an empirical formula for estimating apparent specific heat of food at temperatures belowT_{sh} [cal/$C^{\circ(n_c-1)}$g)].
F	Geometric factor for radiative heat exchange.
Fo_t	$= \alpha_r t_t / \ell^2$. Dimensionless thawing time.
h_m	Convective surface mass transfer coefficient [g of water/(hr cm^2kPa)].
h_q	Convective surface heat transfer coefficient (cal/(hr cm^2 C°)].
k_l	Thermal conductivity of food at T_{sh}[cal/(hr cm C°)].
k_r	Reference thermal conductivity of food. This is a constant in an empirical formula for estimating the thermal conductivity of food at temperatures below T_{sh}[cal/hr cm C°)].
L_r	Latent heat of sublimation of food moisture at −40°C, which is the reference initial temperature of food (cal/g).
ℓ	Characteristic dimension of food. For rectangular food, it is the y-directional side dimension. For cylindrical food it is the radius (cm).
ℓ_v	Food dimension perpendicular to l-direction. For rectangular food, it is the x-directional side. For cylindrical food it is the height (cm).
M_d	$= S_d(T_{or} - T_{ar})/\rho_r$. Constant in an empirical formula for estimating dimensionless density at temperatures below T_{sh}.
M_{ksx}	$= S_{ksx}(T_{or} - T_{ar})/k_{rx}$. Constant in an empirical formula for estimating dimensionless, x-directional thermal conductivity at temperatures below T_{sh}.
M_{klx}	$= S_{klx}(T_{or} - T_{ar})/k_{rx}$. Constant in an empirical formula for estimating dimensionless, x-directional thermal conductivity at temperature above T_{sh}.

M_{kly} = $S_{kly}(T_{or} - T_{ar})/k_{rx}$. Constant in an empirical formula for estimating dimensionless, y-directional thermal conductivity at temperatures above T_{sh}.

n_c Dimensionless constant in empirical formula for estimating apparent specific heat of food at temperatures below T_{sh}.

NR = $\sigma F \epsilon \ell (T_{or} - T_{ar})^3/k_{rx}$. Dimensionless radiative heat exchange conductance.

P_j Arbitrary dimensionless parameter.

P_r Reference water vapor pressure. This is saturation water vapor pressure at -40°C.

R_c = C_l/C_r. Dimensionless specific heat of food at temperatures above T_{sh}.

R_d = $\rho_l/\rho_r - 1$.

R_{kx} = $k_{lx}/k_{rx} - 1$.

s absolute value of upper or lower limit of design variable.

Sf = ℓ_v/ℓ. Shape factor

S_d Constant in empirical formula for estimating food density at temperatures below T_{sh} [(g/cm^{3}C°)].

S_{klx}, S_{kly} Constants in empirical formula for estimating x-directional and y-directional thermal conductivity of food at temperatures above T_{sh} respectively [cal/(hr cm C°^{2})].

S_{ksx} Constant in empirical formula for estimating x-directional thermal conductivity of food at temperatures below T_{sh} [cal/(hr cm C°^{2})].

T Temperature (°C).

T_{or} Reference initial temperature of food. For the present work, $T_{or} = -40$°C.

T_{ar} Reference surrounding convective heat exchange medium temperature. For the present work, $T_{ar} = 8$°C.

t_t Thawing time (hr.)

U = $(T - T_{ar})/(T_{or} - T_{ar})$. Dimensionless temperature. For the present paper, $U = (8 - T)/48$.

X_j Design variable obtained by transforming P_j.

X Design variable obtained by transforming significant dimensionless parameter. A subscript appended to X identifies a coresponding dimensionless parameters.

x,y Coordinate variables. For rectangular food, the x-direction is perpendicular to the l-direction. For cylindrical food, it is the radial direction. The y-direction is the l-direction for rectangular food and is the axial direction for cylindrical food.

α_r = $K_r/(C_r\rho_r)$. Reference thermal diffusivity of food.

γ = k_{ry}/k_{rx}

$\triangle$ Absolute error of parameter identified by symbol placed after $\triangle$.

ϵ Absorbance of radiative heat.

ρ_l Density of food at temperatures above T_{sh}(g/cm3).

ρ_r Reference density. This is a constant in an empirical formula for estimating food density at temperatures below T_{sh}(g/cm3).

σ Stefan-Boltzmann constant [cal/(hr cm^2°K^4)].

ϕ_a Relative humidity of surrounding air. When a surrounding heat exchange medium is liquid, set $h_m = 0$. In this case, any value may be assigned to ϕ_a since this is not used for computation.

Subscripts

a Surrounding convective heat exchange medium or value related to U_a which is a dimensionless surrounding medium temperature.

eh value related to C_{eh}.

f Final temperature at the thermal center of food or value related to U_f which is a dimensionless final food temperature.

frq, max, min Frequently observed, maximum, and minimum values of dimensionless parameters respectively.

in Value related to Bi_n which is Biot number applicable to the surfaces identified by subscript n.

iu Value related to Bi_u, which is Biot number applicable to the surfaces identified by subscript u.

n values applicable to one x-directional side of rectangular food or to one bottom end of cylindrical food.

o value at zero time.

p Radiative heat source.

sf Value Related to Sf.

sh Initial freezing point or temperature at which freezing initiates or thawing completes.

sw Freezing point of pure water. For example $T_{sw} = 0$°C.

u values applicable to all surfaces not associated with subscript n.

uh Value related to U_{sh}, which is a dimensionless initial freezing point.

x,y x- and y-directional values respectively.

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A Computer Aided Method for the Rheological Characterization and Classification of Solid Food Materials

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ABSTRACT

Selected solid food materials were characterized by a modified general Maxwell model in which the elements have predetermined and fixed relaxation times but their number continuously changes with the strain. The fit criterion for the model was its consistency with results obtained in independent tests (e.g. relaxation data at different strains). The properties of the model and the computer programs for the determination of its parameters were tested and evaluated by simulated stress-strain and stress-relaxation curves and by varying the assigned values of the relaxation times.

INTRODUCTION

THE BEHAVIOR of solid foods in deformation, stress relaxation and creep has traditionally been quantified and analyzed by phenomenological-rheological models, frequently presented as mechanical analogs. The constants of these models, as well as their general mathematical structure, are primarily determined on the basis of a preconceived model, usually adapted from the polymer literature. Thus, it is quite common to find stress-relaxation data presented in the form of a discrete linear Maxwellian model and creep data as a discrete linear model of the Kelvin-Voigt type. Such models usually have three to eight characteristic constants or, in model terminology, from one to four relaxation or retardation times, determined by numerical or graphical curve fitting methods. From a rheological viewpoint the application of such linear models is only permissible when the deformations that are involved are very small. The rheology of small deformations, however, is relevant only to a limited number of food problems since most of the situations or events that are related to texture evaluation usually involve large deformations.

Rheological analysis of large strains requires nonlinear models, usually of considerable conceptual and mathematical complexity (e.g. Lockett 1972), that make them unattractive for food applications. The problem is further aggravated by the fact that many food materials are not only structurally nonuniform and nonisotropic but also physically unstable (as a result of enzymatic or microbial activity, for example). These features, in most cases, cannot be properly accounted for in a model without rendering it impractical as a result of the added complexity. They also make it very difficult to measure the pertinent rheological parameters experimentally with an acceptable degree of accuracy and reproducibility. Under these circumstances, it is only natural that the most commonly encountered rheological models for foods are "compromise models." They are based on a linear model skeleton to account for viscoelasticity but modified with special elements such as St. Venant, fracture, contact, stress generator, etc., to account for features such as plasticity, yielding or hardening that cannot be described in terms of linear models. Their main characteristic is that they can describe, qualitatively at least, a multitude of rheological behaviors in certain tests while remaining relatively simple mathematically (Peleg, 1984). Usually, however, the simpler the model the less it can account for a rheological behavior which is outside the range of the experimental conditions under which it was determined.

Furthermore, since there are no generally accepted guidelines to the selection of the model format, there are numerous published models which differ in both their structure and the magnitude of their constants. Consequently, meaningful comparison between the behavior of materials described by different models is virtually impossible. If, however, a generalized model could be constructed so that the difference between materials would only be reflected in the model constants but not in the model structure, then meaningful comparisons would become possible. The main question is whether such a model can be devised at all and, if so, can it be formulated in such a way that it will have manageable mathematical complexity. Even if the answer to these questions is affirmative, it will still be a matter of subjective judgment to determine how much accuracy can be compromised for the sake of mathematical simplicity, and to select between models if more than one is equally appropriate.

The purpose of this study is to develop such a modeling approach, and to analyze its mathematical aspects when applied to rheological characterizations and classification of solid food materials on the basis of their prefailure behavior.

MODEL CONCEPT

THE STRUCTURE of the model's mechanical analog is shown schematically in Fig. 1. This model is basically a generalized Maxwell model but with the following significant modifications: (1) Its elements are divided into groups each having a preselected (and fixed) relaxation time; (2) The number of element groups with different relaxation times, including an elastic group, is also preselected. (In the model shown in Fig. 1 there are n "types" or groups of Maxwell elements (subscript Mi) and an elastic group (subscript E); (3) All the elastic constants of the elements (K's) have a unit stress value so that the viscous element magnitude is also the relaxation time by definition; (4) The number of elements in each group (i.e. NE, and the NM_i) can and does vary with the strain; (5) Any type or group of elements can be eliminated by reaching a critical stress (σ_C) or strain (ϵ_C).

Formulated in this way the model can be considered a simplified version of the model proposed by Drake (1979) with two important distinctions. One is that before disengagement the number of active elements vary in a continuous fashion and the other is that the number of active elements can also increase with the strain, thus accounting for stiffening as a result of strain hardening or compressibility. Mathematically, the response of the model as presented in Fig. 1 is governed by the differntial equation (Peleg, 1977; Normand et al., 1984).

$$d\sigma_T = N_E(\epsilon)K_E d\epsilon + \sum_{i=1}^{n}\left[N_{Mi}(\epsilon)\left(K_{Mi}d\epsilon - \frac{\sigma_{Mi}}{\tau_{Mi}}dt\right)\right] \quad (1)$$

where σ_T is the total stress, ϵ the strain, K_E and K_{Mi} elastic

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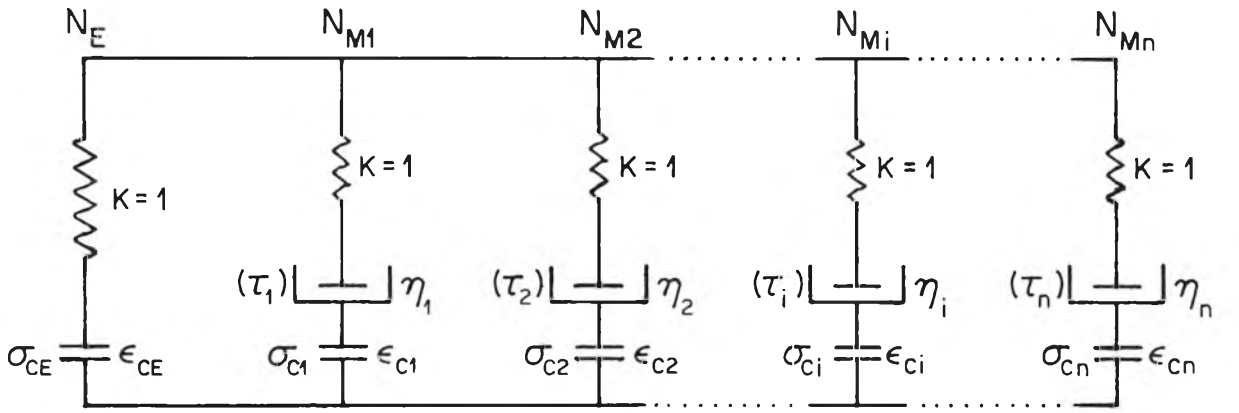

Fig. 1—A generalized Maxwell model with a variable number of elements. Note that all the elastic components have a unit magnitude and that the relaxation times are preselected. N_E is the momentary number of elastic elements, and N_{Mi} the momentary number of Maxwell elements having the same relaxation time. The K's and η's are the elastic and viscous constants of the elements, respectively. Since, however, the elastic constants received unit value the relaxation time of each element (τ_i) had the same numerical value as the corresponding viscous constant (i.e. $\tau_i = \eta_i$). σ_c and ϵ_c are, respectively, the critical stress and strain at which a group of elements can be disengaged.

constants, η_{Mi} viscous constants, σ_{Mi} the monetary stresses supported by each Maxwell element, and N_E and N_{Mi} the mometary numbers of active elements in elastic and Maxwellian states respectively.

For creep, Eq. (1) can be rearranged to yield (Peleg, 1979):

$$\frac{d\epsilon}{dt} = \frac{\frac{d\sigma_T}{dt} + \sum_{i=1}^{n} \frac{\sigma_{Mi}}{\tau_{Mi}}}{N_E(\epsilon)K_E + \sum_{i=1}^{n} N_{Mi}(\epsilon)K_{Mi}} \quad (2)$$

In stress relaxation, the number of elements is fixed and therefore (Peleg, 1977):

$$\sigma_T(t) = N_E(\epsilon)K_E + \sum_{i=1}^{n} N_{Mi}(\epsilon)\sigma_{0Mi}\exp\left(-\frac{t}{\tau_{Mi}}\right) \quad (3)$$

Gross failure in terms of the model is reached when all or most of the elements are fractured or disengaged. The discussion, however, will only address the prefailure rheological behavior.

The initial number of elements in each group, as well as their change as a function of the strain, cannot be known a priori. Qualitatively, however, it is clear that yielding or internal fracture as a result of structural disintegration will be expressed as a decrease in the number of elements while stiffening will be expressed, as previously mentioned, by an increase. An alternating pattern, i.e. stiffening followed by yielding or vice versa is, of course, also possible. One of the simplest mathematical forms for representing these patterns is (Peleg, 1984):

$$N(\epsilon) = N_o(1 - A\epsilon)^n \quad (\text{"yielding"}) \quad (4)$$

$$N(\epsilon) = N_o(1 + A\epsilon)^n \quad (\text{"stiffening"}) \quad (5)$$

$$N(\epsilon) = N_o(1 - A_1\epsilon^{n1} + A_2\epsilon^{n2}) \quad (\text{"alternating"}) \quad (6)$$

where $N(\epsilon)$ is the momentary number of elements of a particular group at strain, and the A's and n's are constants.

It was previously demonstrated (Normand et al., 1984) that the described model can be written in the form of a computer program, and that with the selection of appropriate sets of constants, almost all known rheological behavior patterns can be simulated. It was also demonstrated that the model concept, with some simplifying assumptions, can be used to predict failure conditions with reasonable accuracy from stress relaxation and creep data (Peleg, 1979; Finkowski and Peleg, 1981). The main drawback of the model as formulated is that there are still no clear guidelines for the selection of element relaxation times, the initial number of elements in each group and the exact mode of their change with the strain (and also possibly with the stress or time). Furthermore, the construction of a model of the proposed kind and its application to the behavior of a particular food system under any test within a desired accuracy level is not by itself a proof of the model's uniqueness. In other words, even if one model works, it is quite possible that the selection of different constants (e.g. the relaxation times and the number of element groups) will yield similar results. If this is indeed the case, a rational approach needs to be developed for the selection of an optimal model in light of practical as well as rheological considerations and keeping in mind that an element of arbitrariness cannot be totally avoided.

If it can be shown that within reasonable accuracy requirements the optimal or even another compromise model is applicable to all solid foods, then such a model can become a tool for universal classification of solid foods. This is because once the relaxation times and number of groups have been established, every material will be characterized only by the initial number of elements in each group and their mode of change with the strain. For example, if it can be established that a successful model can be constructed by a three group array (i.e., an elastic and two Maxwellian groups) and the simplest change modes (compare with Eq. 2–4):

$$N(\epsilon) = N_o(1 \pm A\epsilon) \quad (7)$$

then the prefailure behavior of all food materials to which this model applies can be characterized by six constants only, namely $N_{E(0)}$, $N_{M1(0)}$, $N_{M2(0)}$, A_E, A_{M1} and A_{M2}. It is obvious from the model structure that the absolute magnitudes of $N_{E,0}$, N_{M1} and N_{M2} represent the overall stiffness of the material, and their relative magnitudes represent the general rheological features of the material. The constants A_E, A_{M1} and A_{M2} represent the deviation from linear viscoelasticity (i.e. if all the A's have zero value the food is supposed to behave as a linear viscoelastic material) and whether the material is compressible or strain hardened (positive values) or crumbly and yielding (negative values).

MATERIALS & METHODS

FORCE-DEFORMATION and relaxation curves of different food materials recorded by an Instron Universal Testing machine (model TM) were used as the data source. A complete set of data included force deformation curves at a given crosshead speed and at least three force-relaxation curves of the same material obtained at different defor-

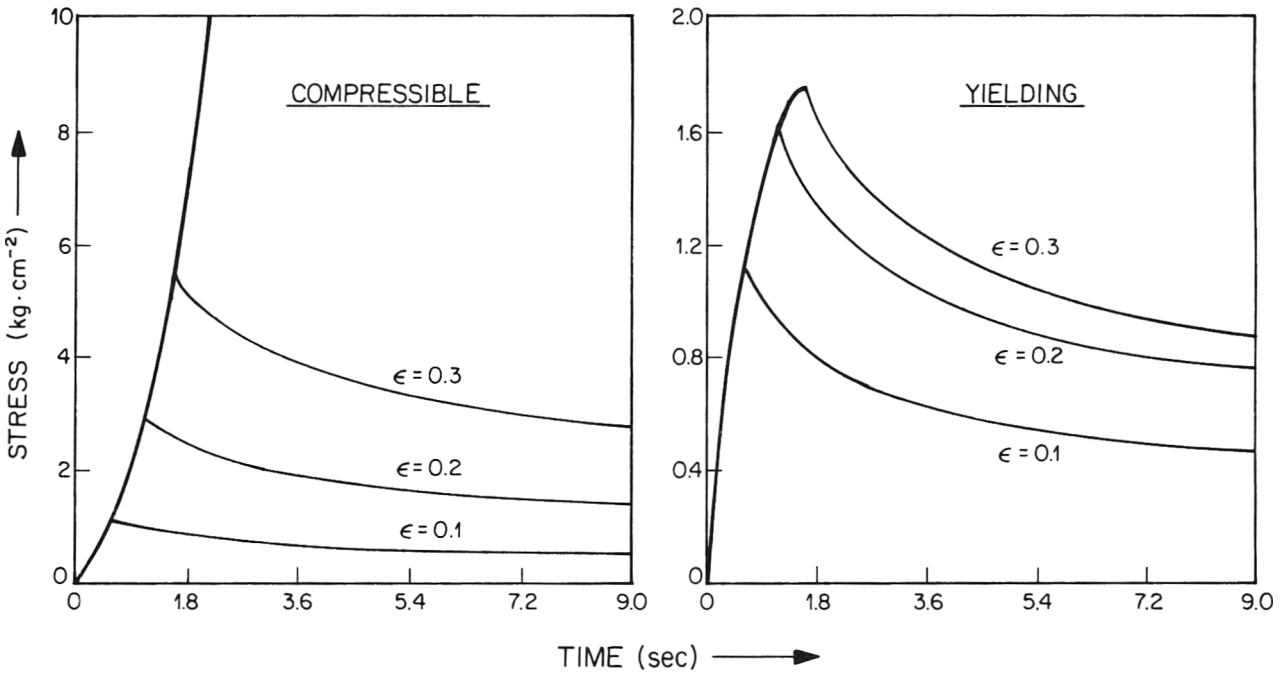

Fig. 2—Examples of simulated stress-time and stress-relaxation curves of a compressible material (left) and a yielding material (right). The stress time curves were generated for a specimen 1 cm in length subjected to a constant deformation rate of 0.1667 cm.sec⁻¹ (10 cm min⁻¹). The relaxation curves were generated at strains of 0.1, 0.2 and 0.3. (The model used for the compressible material was a five element array with $N_{E0} = N_{M10} = N_{M20} = 3$. $\tau_1 = 1$ and $\tau_2 = 5$ sec and all the K's 1.0 kg.cm⁻². Its mode of change was governed by $N = N_0 (1+2\epsilon)^2$. The model for the yielding material was also a five element with $N_{E0} = N_{M10} = N_{M20} = 5$, $\tau_1 = 1$ and $\tau_2 = 5$ sec and all the K's 1.0 kg.cm⁻². Its mode of change was governed by $N = N_0 (1-\epsilon)^2$.

mation levels which had been reached by the same speed. Since textural nonuniformity can become a factor, similar data were also generated using the simulation program described by Normand et al. (1984). In the case of the simulated data, the model constants were known a priori, a factor that facilitated the evaluation of the modeling method and its sensitivity.

Data processing

Both the simulated and the recorded curves were digitized using a graphics tablet (HIPAD, Houston Instrument, Inc., Houston, TX) connected to an Apple II computer. The data files so created were transformed into stress-time relationships and were passed for further processing by a Cyber mainframe computer at the University Computing Center. The analysis included the following: (1) Fitting the data with different models to test the methodology sensitivity; (2) Fitting the data using specially modified statistical procedures (based on the standard BMDP and ANL packages) to test whether the latter can effect the magnitude of the calculated parameters.

In all the cases the fit criterion was that the constants calculated from two or more sets of data (e.g. two relaxation curves) could be used to predict the other data set (e.g. the stress-strain relationship and/or another relaxation curve at a different strain).

The results of the model as well as the original data were later replotted to allow for visual comparison.

RESULTS & DISCUSSION

Analysis of simulated stress-strain and stress-relaxation curves

In order to assess the proposed method's performance, two preliminary analyses were performed. The first was to establish the accuracy and reproducibility of the digitizing step and the second to test the sensitivity of the statistical procedures. As could be expected (Kaletunc-Gencer and Peleg, 1984) the digitizing process was very reproducible. This was evident from the fact that curves replotted using digitized data were practically indistinguishable from the original curves. The applicability of the statistical and calculation procedures is demonstrated in Table 1 where the values of the original model parameters and those calculated from the corresponding simulated curves are compared. Although the values are fairly close, particularly in the simpler models, they deviated on the order of up to about 20% from the more complicated models. In all the cases, a deviation from the expected value of the initial number of elements (N_o) was "compensated" for by a corresponding increase or decrease in the value of the element's change mode in the particular group (i.e. the A's in Eq. 4 and 5).

Although the discrepancy between the original and retrieved model parameters indicates that the method has only limited accuracy, it is also clear from the comparison that the general rheological character of the original model can be retrieved unambiguously. This conclusion, however, may be invalid if the preselected relaxation times have similar values. For such models a deviation from the value of the initial number of elements in one group can be compensated for by a change in the overall number of elements in the other groups. Since the proposed classification method is based on a limited number of *representative* relaxation times, the selection of elements with similar relaxation times should naturally be avoided.

The simulation as well as the experimental data shown below were tested by varying the sought model's mathematical structure and by applying two different statistical packages. The two statistical procedures yielded similar, but not always identical, constants. This was mainly a result of the inherent ambiguity of the model with respect to the magnitude of the initial number of elements (N_o's) and their change mode parameter (the A's in Eq. 4 and 5). The differences, however, were relatively small so that both packages would be equally useful. Naturally, it was also learned that the model was fairly insensitive to the power n in Eq. 4 and 5, and, therefore, all subsequent data analyses were processed using the mode change expressed by Eq. 7 (i.e. with n = 1).

Analysis of experimental data

Stress-time relationships in deformation and stress relaxation at three strains of apple, potato, squash and turnip flesh are

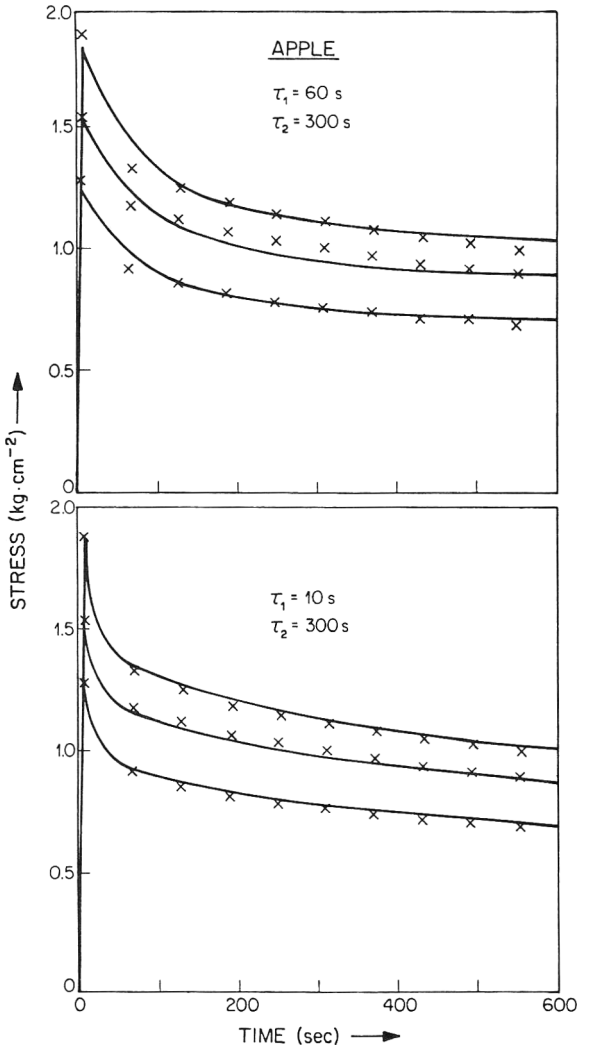

Fig. 3—Experimental and fitted data of apple flesh using a "five element" model with relaxation times of 60 and 300 sec (top) and 10 and 300 sec (bottom). Solid lines are fitted curves and the X is experimental data points. The change mode of the element numbers is given in Table 2. The three relaxation curves were determined at strains of 0.1, 0.2 and 0.3.

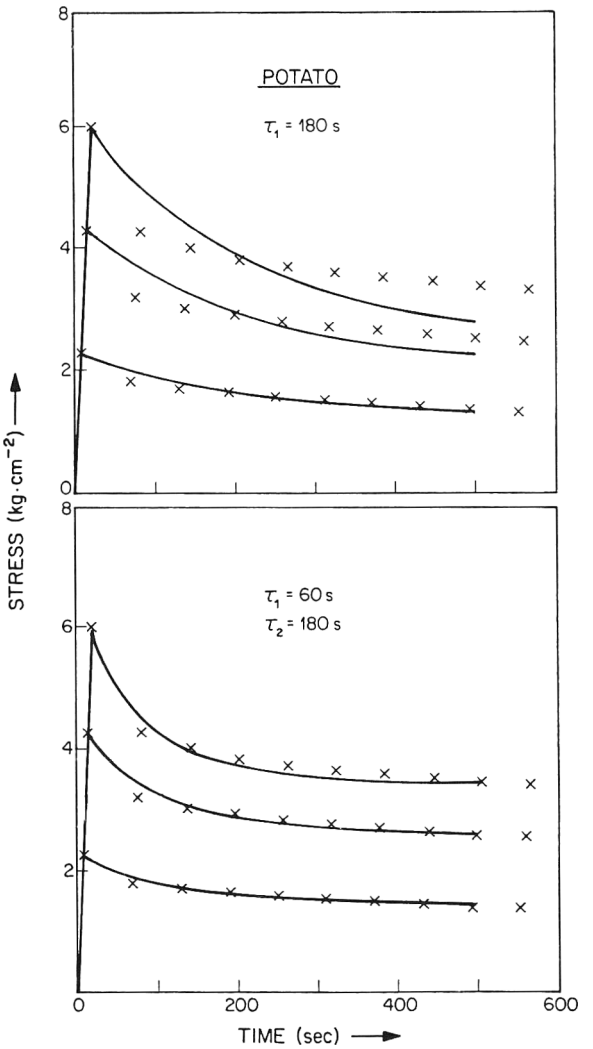

Fig. 4—Experimental and fitted data of potato flesh using a "three element" model with relaxation time of 180 sec (top) and a "five element" model with a relaxation time of 60 and 180 sec (bottom). Solid lines are fitted curves and the X is experimental data points. The change mode of the element numbers is given in Table 2. The three relaxation curves were determined at strains of 0.1, 0.2 and 0.3.

shown in Fig. 3 to 6. The figures also show the fitted relationships using models with one or two preselected relaxation times. They demonstrate that the goodness of fit was practically independent of the selected relaxation times, provided of course that they were more or less representative. This was particularly the case with respect to the longer relaxation times (i.e. from 180 sec and up). The latter was mainly due to the predominantly solid character of the materials and therefore especially noticed in squash and turnip flesh (Fig 5 and 6) where the contribution of these elements to the total stress was on the order of 15 and 25%, respectively). The corresponding model's calculated parameters are summarized in Table 2. The table is a clear indication that the model, despite its crudeness and inherent ambiguities, captured the main rheological characteristics of the tested materials with respect to both stiffness (total numbers) and the dominant feature of their relaxation time spectrum. It also demonstrates that a reasonable characterization of such materials can be done by using three groups of elements and, in a few cases, only two. One ought to be aware though that the selection of the representative elements, or the model's relaxation times may affect the model interpretation. Thus, the models reached for all the materials reported in this work (Table 2) clearly indicate that they are "yielding," i.e that their total number of elements, particularly the elastic and those having long relaxation times were progressively decreasing with the strain. The change in the rheological behavior, however, was also manifested in an increase in the number of elements with short relaxation times (i.e. 10 sec for apple flesh and 60 sec for the others). This can be interpreted as a transformation toward more "liquid" properties, a phenomenon that is clearly evident from the increasing decay rate at the initial stages of the stress relaxation. Since this transformation in rheological behavior will most likely be reflected in the term representing the elements with the shortest relaxation time, comparison between different materials may not be meaningful unless the models chosen for their presentation have the same number of elements and the same relaxation times.

It should also be added that the model, as shown, has certain inherent ambiguities and limited accuracy. Therefore, the number of digits, as reported in Tables 1 and 2, is clearly in excess of the number of significant digits. Rounding off the model coefficients can be done either after the calculation has been completed or by imposing a limit on the number of digits in the program itself. This was tried and did not result in any appreciable modification in the model's features.

Since the experimental data presented in this work were

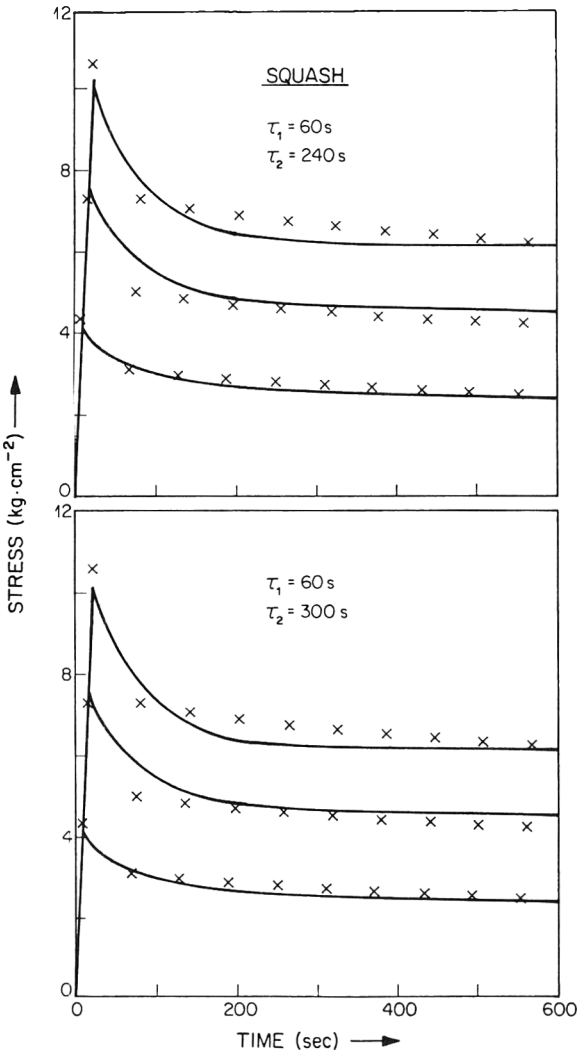

Fig. 5—Experimental and fitted data of squash flesh using a "five element" model with relaxation times of 60 and 240 sec (top) and 60 and 300 sec (bottom). Solid lines are fitted curves and the X is experimental data points. The change mode of the element numbers is given in Table 2. The three relaxation curves were determined at strains of 0.1, 0.2, and 0.3.

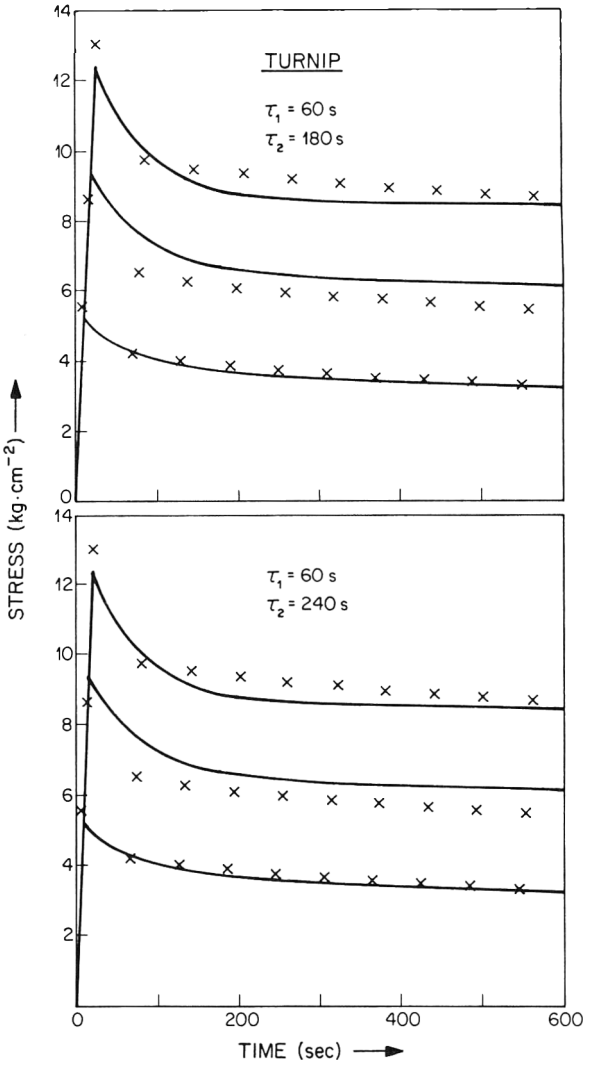

Fig. 6—Experimental and fitted data of turnip flesh using a "five element" model having relaxation times of 60 and 180 sec (top) and 60 and 240 sec (bottom). Solid lines are fitted curves and the X is experimental data points. The change mode of the element numbers is given in Table 2. The three relaxation curves were determined at strains of 0.1, 0.2 and 0.3.

Table 1—Calculation of the model constants from simulated stress-strain and stress-relaxation curves, digitized with a graphics tablet.[a]

Model	Original expression	Calculated expressions
Three Element Yielding	$N_E = 70(1-\epsilon)$ $N_M = 20(1-\epsilon)$	$N_e = 69.9(1-0.99\epsilon)$ $N_M = 19.6(1-0.93\epsilon)$
Three Element Compressible	$N_E = 4(1+\epsilon)^2$ $N_M = 4(1+\epsilon)^2$	$N_e = 4.09(1+0.96\epsilon)^2$ $N_M = 3.80(1+1.17\epsilon)^2$
Five Element Yielding	$N_E = 5(1-\epsilon)^2$ $N_{M1} = 5(1-\epsilon)^2$ $N_{M2} = 5(1-\epsilon)^2$	$N_E = 4.99(1-1.06\epsilon)^2$ $N_{M1} = 5.43(1-1.16\epsilon)^2$ $N_{M2} = 4.64(1-0.78\epsilon)^2$
Five Element Compressible	$N_E = 3(1+2\epsilon)^2$ $N_{M1} = 3(1+2\epsilon)^2$ $N_{M2} = 3(1+2\epsilon)^2$	$N_E = 3.06(1+2.39\epsilon)^2$ $N_{M1} = 2.6(1+2.43\epsilon)^2$ $N_{M2} = 3.63(1+1.69\epsilon)^2$

[a] For the model structure and nomenclature see Fig. 1 and Eq. (1), (4) and (5).

Table 2—Classification of selected food materials using a Maxwellian model with elements having preselected relaxation times[a]

Material	N_E	τ_1 and N_{M1}	τ_2 and N_{M2}
Apple Flesh	13.75(1 − 2.69ϵ)	60 sec 7.11(1 − 1.79ϵ)	300 sec 3.79(1 − 2.64ϵ)
	13.09(1 − 2.69ϵ)	10 sec 5.51(1 + 1.41ϵ)	300 sec 6.42(1 − 2.53ϵ)
Potato Flesh	14.6(1 − 0.89ϵ)	180 sec 9.05(1 − 0.44ϵ)	– (3 element model)
	14.79(1 − 0.73ϵ)	60 sec 0.78(1 + 48.5ϵ)	180 sec 8.13(1 − 2.68ϵ)
Squash Flesh	27.99(1 − 0.829ϵ)	60 sec 14.06(1 + 0.384ϵ)	240 sec 3.99(1 − 3.33ϵ)
	27.64(1 − 0.803ϵ)	60 sec 14.26(1 + 0.274ϵ)	300 sec 4.33(1 − 3.33ϵ)
Turnip Flesh	36.59(1 − 0.719ϵ)	60 sec 13.66(1 + 0.304ϵ)	180 sec 8.48(1 − 3.33ϵ)
	35.92(1 − 0.668ϵ)	60 sec 14.52(1 + 0.062ϵ)	240 sec 8.33(1 − 3.33ϵ)

[a] For the model structure and nomenclature, see Fig. 1 and Eq. (1) and (6).

obtained from texturally uniform and fairly isotropic materials, and from specimens with similar dimensions, the calculated parameters, by definition, cannot reflect textural variability or specimen dimension effects. The latter can be accommodated by presenting the model parameters as a function of the specimen dimensions, or as a range reflecting the textural variability. In both cases, the range will be meaningful for comparison because it refers to the same fixed relaxation times. It should also be remembered that the model on which the classification method is based is a phenomenological model, and that the

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HPLC Determination of Carotenoids in Fruits and Vegetables in the United States

JANICE L. BUREAU and RODNEY J. BUSHWAY

ABSTRACT

A study was conducted to determine alpha- and beta-carotene and beta-cryptoxanthin in twenty-two fruits and vegetables. Foods were obtained from wholesale distributors from five locations throughout the United States (Los Angeles, Dallas, Chicago, Miami, and Boston) three times during a year (November, March, and July). Mean vitamin A activity in retinol equivalents (RE) of each food along with average values for the individual carotenoids (μg/100g) are given. beta-Carotene was the most prevalent carotenoid. Carrots, beet greens, spinach, swiss chard and sweet potatoes had the most vitamin A activity. Analysis of variance indicated that there were no significant differences among either locations or time of analysis. A comparison was made between the new values and the ones listed in USDA Handbook No. 8 and revised Handbook No. 8-9. There were differences between the old and new values in 14 of the 22 fruits and vegetables analyzed.

INTRODUCTION

AS RESEARCHERS learn more about the relationship of dietary intake and human health, an accurate assessment of the nutrient content of foods is becoming more important. One such nutrient is vitamin A. Recent scientific work has demonstrated that some vitamin A active carotenoids have anticancer and antiulcer properties.

Epidemiological studies have shown that an inverse relationship exists between the risk of cancer and the consumption of foods containing certain carotenoids (Shekelle et al., 1981; Moon and Itri, 1984; Ong and Chytil, 1983; Colditz et al., 1983). Several laboratory studies have shown inhibition of cancer cell lines and actual tumor regression in animals by carotenoids (Burton and Ingold, 1984; Mathews-Roth, 1982; Seifter et al., 1983; Wattenburg, 1983). Antiulcer properties were observed by Javor et al. (1983). It was shown that retinol and several carotenoids (beta-carotene, betacryptoxanthin, zeaxanthin, lutein, capsorubin, capsanthin, lycopene and capsanthol) were involved in the cytoprotective injury of the gastric mucosa.

Furthermore, it is widely known that food composition tables are invaluable tools for nutritionists when planning and evaluating diets and establishing dietary guidelines (Goddard and Matthews, 1979). To date, the tables used for fruits and vegetables are usually ones like the U.S. Department of Agriculture's Handbook No. 8 (Watt and Merrill, 1963) or revised Handbook No. 8-9 (Gebhardt et al., 1982), neither of which includes individual carotenoid values. Also, the methods employed for obtaining the vitamin A values in these tables, for the most part, are not the current analytical procedures.

The purpose of this study was to employ high performance liquid chromatography to quantify the major vitamin A active compounds and total vitamin A activity of some of the most frequently consumed fruits and vegetables purchased from five locations throughout the United States, three times during a year.

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MATERIALS & METHODS

Reagents

Trans alpha- and beta-carotene standards were obtained from Sigma Chemical Co. (St. Louis, MO). beta-Cryptoxanthin was obtained from Hoffmann-LaRoche (Basel, Switzerland). Magnesium carbonate (anhydrous powder) and sodium sulfate (anhydrous granular form) and all solvents were purchased from Fisher Scientific Co. (Fair Lawn, NJ). Tetrahydrofuran stabilized with butylated hydroxy toluene (BHT), chloroform and petroleum ether were ACS grade, while the acetonitrile and water were HPLC grade.

Sampling procedure

In order to obtain data representative of fresh foods available to consumers across the United States, fruit and vegetable samples were obtained from five cities (Los Angeles, Dallas, Chicago, Miami and Boston), three times during a year (November, March, and July). This sampling method accounts for geographical, seasonal, cultivar and handling conditions in obtaining the values for vitamin A active carotenoids. The following foods were analyzed: apricots, asparagus, beet greens, blueberries, broccoli, cantaloupe, carrots, green beans, greenpepper, grapefruit, lettuce, nectarines, okra, oranges, peaches, peas, raspberries, spinach, squash, strawberries, sweet potato and swiss chard. The foods were shipped by air to the laboratory (one crate per item). All foods were removed from the containers and sampled three times. These samples (1–2 kg each) were chopped into small pieces and a 10g subsample was removed from each of the three samples. All foods were extracted immediately upon arrival unless they came in late at night, in which case they were refrigerated until morning.

High-performance liquid chromatography

The HPLC equipment consisted of a Waters Model ALC/GPC 254 Liquid Chromatograph with a Model 6000A solvent delivery system, a Model U6K injector (Waters Associates, Milford, MA), a Schoeffel variable wavelength detector (Westwood, NJ), and on Omni-Scribe recorder (Houston Instruments, Austin, TX). The operating conditions employed for provitamin analysis were those of Bushway and Wilson (1982) and included a Partisil 5 ODS column 25 cm × 4.6 mm ID (Whatman, Inc., Clifton, NJ) along with a solvent system of acetonitrile-tetrahydrofuran-water (85:12.5:2.5) pumped at a flow rate of 2.0 mL/min. Visible detection was achieved at 470 nm (0.04 Absorbance Units Full Scale A.U.F.S.)

Preparation of carotenoid standards

Stock solutions of trans alpha- and beta-carotene were prepared by weighing 25 mg of each into separate 100 mL low actinic volumetric flasks. The flasks were brought to volume with stabilized tetrahydrofuran. Aliquots of 0.5, 1.0, 1.5 and 2.0 ml were removed from each and placed into four 50 mL low actinic volumetric flasks. A stock solution of beta-cryptoxanthin was prepared by weighing 12 mg into a 100 mL low actinic volumetric flask and brought to volume with stabilized tetrahydrofuran. Aliquots of 1.0, 2.0, 2.5 and 3.0 mL were removed from the beta-cryptoxanthin stock solution and placed intothese four 50 mL low actinic volumetric flasks. These working standards were brought to volume with stabilized tetrahydrofuran. Ten microliters of each standard were injected, and quantification was performed using peak height.

Determination of standard purities

The purities of alpha-carotene and beta-carotene were determined spectrophotometrically on a Beckman (DU8 Beckman Instruments,

Fullerton, CA). Extinction coefficients used were: (E 1% cm) alpha-carotene, 2800 at 444nm in petroleum ether; beta-carotene, 2396 at 465nm in chloroform. The purity of the beta- cryptoxanthin standard was 95% as determined by Hoffman LaRoche (Basel, Switzerland).

Extraction of vitamin A active compounds from fruits and vegetables

Carotenoid extraction was achieved by employing the method of Bushway and Wilson (1982). Triplicate 10g samples of each of the fruits and vegetables were extracted with 5.0g anhydrous Na_2SO_4, 1.0g $MgCO_3$ and 125 mL tetrahydrofuran in a 1L Waring Blendor at a moderate speed for 5 min. The extract was vacuum-filtered through a Buchner funnel fitted with Whatman No. 42 filter paper. The filter cake was re-extracted to remove all the carotenoids. The filtrates were combined and brought to a 500 mL volume with tetrahydrofuran. For all the vegetables and cantaloupe, a 100 mL aliquot of each sample was transferred to a 250 mL round bottom flask and evaporated to dryness using a Bushi rotary evaporator at a temperature of 40°C. For all other fruits, the combined extracts were entirely evaporated. All evaporated samples were redissolved in 10 ml of tetrahydrofuran with the aid of sonication. The extracts were transferred to 20 mL scintillation vials and placed in a −20°C freezer until injection into the HPLC.

Determination of Vitamin A Activity by HPLC

The HPLC was calibrated daily for carotenoids by injecting 10 μl of each working standard in duplicate. Ten to 25 μL of each sample extract were injected for carotenoid analysis, depending on the concentration. Peak height was employed for quantification.

Statistics

The data were analyzed by the analysis of variance method to test for significant differences among either locations or months of analyses. A t-test was employed to determine significant differences among our values and values listed in USDA Handbooks No. 8 and No. 8–9. A 0.05 level of significance was used for all tests. The data were processed by using the Statistical Analysis System package (SAS, 1982).

RESULTS & DISCUSSION

THE HPLC METHOD of Bushway and Wilson (1982) was used in this study because it is quick, accurate and reproducible compared to the AOAC (1984) method for carotenoid analysis. At the time that the HPLC procedure was developed a comparison was performed with the AOAC method and it was shown that the HPLC technique was far superior for carotenoid analyses.

The average vitamin A activities of the fruits and vegetables using HPLC are given in Table 1. These means are comprised of the total activity from alpha-carotene, beta-carotene and beta-cryptoxanthin. From Table 1, it can be seen that beet greens, carrots, spinach, sweet potatoes, and swiss chard were the best sources for provitamin A activity, while broccoli, cantaloupe, okra, peas, asparagus, apricots, and green beans were good supplemental sources of vitamin A active carotenoids.

There were a large variation in vitamin A activity for many of the foods (Table 1). This was to be expected, since there were many different cultivars of each commodity along with different handling conditions. Two vegetables that exemplified this variation were the sweet potatoes and lettuce. Sweet potato values ranged from 30–3308 RE since the white, orange and red cultivars were included in these values. Lettuce, was sampled at the outer, middle and inner layers. The outer layer was the greenest and contained the most vitamin A activity, while the inner layer had very little vitamin A active carotenoids.

Finally, an analysis of variance performed over all the foods in Table 1 demonstrated that there were no significant differences among either locations or months of analyses. Therefore, for total vitamin A active carotenoids, it does not matter where you obtain your fruits and vegetables or during what time of the year.

Table 2 shows the distribution of vitamin A activity of the fruits and vegetables over each of the provitamins analyzed.

Table 1—Vitamin A activity from provitamins of twenty-two fruits and vegetables

Product	Sampling Time	n[d]	Mean ± S.D.[a]	Range[a]
Apricots	Nov.[b]			
	March[b]			
	July	6	104.64 ± 47.93	38.17– 161.29
Asparagus	Nov.	9	76.55 ± 18.33	46.60– 108.16
	March	15	114.67 ± 31.38	66.79– 159.65
	July	3	82.27 ± 21.40	63.29– 105.49
Beet Greens	Nov.	12	880.63 ± 209.35	462.96–1297.25
	March	12	875.29 ± 207.33	504.20–1239.32
	July	12	757.29 ± 200.90	327.10–1066.31
Blueberries	Nov.[b]			
	March[b]			
	July	6	2.15 ± 2.55	0.00– 6.01[c]
Broccoli	Nov.	15	105.63 ± 31.26	60.68– 153.51
	March	15	107.95 ± 41.86	106.84–234.59
	July	12	99.77 ± 27.25	61.97– 138.89
Cantaloupe	Nov.[b]			
	March	3	226.49 ± 35.87	199.45– 267.18
	July	6	299.31 ± 57.29	219.53– 369.94
Carrots	Nov.	15	1281.15 ± 643.07	258.12–2643.37
	March	15	1643.26 ± 536.75	1134.21–2529.28
	July	15	1823.20 ± 283.18	1439.36–2345.04
Green Beans	Nov.[b]			
	March	9	77.28 ± 19.35	59.17– 125.92
	July	6	49.27 ± 9.74	33.34– 61.01
Grapefruit	Nov.	27	60.56 ± 18.95	33.85– 96.99
	March	21	32.25 ± 23.39	1.45– 69.44
	July	15	23.08 ± 16.00	0.00– 50.17
Green pepper	Nov.[b]			
	March[b]			
	July	3	35.87 ± 6.10	30.59– 42.55
Lettuce (Iceberg)	Nov.	12	122.90 ± 196.99	16.18– 579.89
	March	15	40.12 ± 18.96	15.27– 79.52
	July	15	16.53 ± 14.26	0.00– 58.02
Nectarines	Nov.[b]			
	March[b]			
	July	3	11.07 ± 1.40	10.12– 12.68
Okra	Nov.	9	65.00 ± 17.75	50.46– 96.77
	March	9	93.69 ± 32.52	58.25– 158.67
	July	3	44.14 ± 15.93	25.84– 54.91
Oranges	Nov.	15	4.19 ± 1.50	1.69– 6.56
	March	21	9.21 ± 5.72	1.45– 25.10
	July	12	14.15 ± 6.60	6.39– 25.14
Peaches	Nov.[b]			
	March	9	5.83 ± 1.06	4.35– 7.94
	July	15	18.59 ± 6.49	7.79– 29.72
Peas	Nov.[b]			
	March	9	102.62 ± 16.18	82.70– 131.56
	July	6	81.56 ± 10.77	63.82– 94.50
Raspberries	Nov.[b]			
	March[b]			
	July	6	7.00 ± 1.00	5.76– 8.07
Spinach	Nov.	12	887.94 ± 352.77	474.91–1554.98
	March	9	833.71 ± 168.27	673.76–1220.76
	July	15	805.87 ± 213.83	380.07–1049.28
Squash (Summer)	Nov.	12	36.03 ± 21.31	10.53– 85.73
	March	9	32.50 ± 8.69	22.27– 48.53
	July	12	23.76 ± 5.09	15.77– 30.47
Strawberries	Nov[b]			
	March	9	3.86 ± 2.22	1.36– 7.82
	July[b]			
Sweet Potato	Nov.	15	1434.19 ± 1047.21	35.09–3276.60
	March	12	1712.42 ± 1182.77	30.70–3308.82
	July	6	882.97 ± 615.41	295.95–1957.37
Swiss Chard	Nov.	3	644.27 ± 91.04	542.12– 716.85
	March	8	1001.91 ± 339.22	637.06–1604.26
	July	6	513.48 ± 236.98	236.49– 818.32

[a] Units are retinol equivalents (RE)/100g sample
[b] Product not available at this time
[c] 0.00—not at a detection limit of 1 μg/100g product for all compounds
[d] n—number of determinations

Table 2—Vitamin A activity from each of the provitamins analyzed

	alpha-Carotene[a]	beta-Carotene[a]	beta-Cryptoxanthin[a]
Apricots	0.00[b]	615.16	22.46
Asparagus	17.41	581.37	0.00[b]
Beet Greens	3.47	5027.67	0.00[b]
Blueberries	0.00[b]	12.91	0.00[b]
Broccoli	1.61	762.89	0.00[b]
Canataloupe	8.99	1642.79	5.85
Carrots	3789.67	7602.78	0.00[b]
Green Beans	64.44	364.23	0.00[b]
Greenpepper	33.78	217.13	0.00[b]
Grapefruit	1.03	248.23	6.72
Lettuce (Iceberg)	4.43	329.86	0.00[b]
Nectarines	0.00[b]	49.22	34.38
Okra	28.09	431.84	0.00[b]
Oranges	19.56	40.04	7.23
Peaches	2.88	76.68	3.39
Peas	16.02	557.16	0.00[b]
Raspberries	0.00[b]	41.96	0.00[b]
Spinach	0.00[b]	4312.01	0.00[b]
Squash	12.13	177.56	0.00[b]
Strawberries	4.52	20.86	0.00[b]
Sweet Potato	0.00[b]	8610.86	0.00[b]
Swiss Chard	57.87	4568.46	0.00[b]

[a] Units are μg/100g product, averaged over all the locations and months of analyses
[b] 0.00—none detected at a detection limit of 1 μg/100g product for all compounds

Table 3—Comparison of total vitamin A activity of the vegetables and fruits analyzed to USDA Handbook No. 8 and No. 8-9 values

Item	Handbook values[a,b]	Analyzed values[a,c]
Apricots	2612	1046
Asparagus	900	983
Beet Greens	6100	8139
Blueberries	100	22
Broccoli	2500	1274
Canataloupe	3224	2750
Carrots	11000	15475
Green Beans	600	661
Grapefruit	259	422
Greenpepper	1540	369
Lettuce	330	554
Nectarines	736	111
Okra	520	743
Oranges	205	88
Peaches	535	1138
Peas	640	895
Raspeberries	130	70
Spinach	8100	8402
Squash	460	306
Strawberries	27	39
Sweet Potatoes	8800	15351
Swiss Chard	6500	7664

[a] Values are given in IU/100g of sample
[b] Values for vegetables are from Handbook No. 8, while values for fruits are from Handbook No. 8-9
[c] Average value for each product, from all locations and months of analyses

With the exception of carrots, beta-carotene comprises 85% of the total provitamin A activity of the vegetables. There was no detectable beta-cryptoxanthin observed in the vegetables while most of the fruits (except strawberries, raspberries, and blueberries) contained some beta-cryptoxanthin. Apricots, grapefruit and cantaloupe were the only fruits analyzed that were good supplemental sources of vitamin A activity.

A comparison of the means of the vitamin A activities that were obtained through HPLC analysis and the USDA Handbook No. 8 and No. 8-9 values can be seen in Table 3. Differences were found for apricots, beet greens, blueberries, broccoli, carrots, grapefruit, greenpepper, nectarines, okra, oranges, peaches, raspberries, squash and sweet potatoes. Of these fourteen foods that differed from the Handbook values nine were lower than the handbook values and five were higher.

There are several factors that can be attributed to these differences in Table 3. The length of storage after harvesting, type of storage, the packaging and handling during shipment, whether the product was mechanically or hand harvested, the cultivars and the method of analysis are the most important factors. One of these factors, the method of analysis, can be easily controlled from laboratory to laboratory. Since the procedure employed can create a lot of variation in values and since there are numerous provitamin A methods used for nutrient data collection, a collaborative study should be performed on the best methods so that a standard procedure could be chosen for all to use in obtaining nutritional values for food tables.

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Snap Bean Texture Softening and Pectin Solubilization Caused by the Presence of Salt during Cooking

J. P. VAN BUREN

ABSTRACT

Special procedures were used to separate the effects on softening and pectin solubilization of the presence of salt during cooking from the effects of salt apart from cooking. The effects of the presence of salt during cooking, calculated with respect to corresponding distilled water cooked beans, were greater with processing conditions of lower blanch temperature and shorter cook times, consequently they were greater with higher canned bean firmness and lower liquor pectin. Neither softening nor pectin solubilization had a significant relation to the reduction in pH induced by NaCl. The softening due to salts was seen with NaCl, KCl, and, to a lesser extent, with $CaCl_2$, and it may be the result of accelerated degradation of pectins.

INTRODUCTION

THE FIRMNESS of cooked vegetables can be modified by the addition of salts. While there have been many studies on the effects of monovalent and divalent salts on cooked vegetable texture, virtually all of them dealing with well cooked foods such as canned vegetables have involved addition of the salts before canning or cooking. (Blair and Ayres, 1943; Lenz and Weckel, 1967; Hughes et al., 1975). It has recently been shown (Van Buren, 1983) that softening caused by NaCl was due to two mechanisms, one requiring heat treatment, and another relatively independent of heat treatment. Thus, earlier results reflect both the cooking and noncooking actions of salts. The salt action during cooking is the subject of the present paper. It can be measured in canned vegetables as the difference between beans cooked in salt solutions and beans treated with salt after cooking (Van Buren, 1983).

Pectin is intimately connected to vegetable firmness (Doesburg, 1965) and is partially solubilized when plant tissues are softened by heating. It seemed reasonable to expect that greater softening caused by the presence of salt during cooking would be accompanied by additional pectin solubilization. This hypothesis was tested in the present research.

A further purpose of this study was to observe the influence of different blanch temperatures and cooking times on the salt cook effects in snap beans and the relation of these effects to the canned bean firmness and liquor pectin. In addition, the ability of different salts to cause softening was examined.

MATERIALS & METHODS

SNAP BEANS were canned in our pilot plant. Pods, 7.3–9.5 mm in diameter and cut to 2.5 cm lengths, were blanched in water for 2 min at the indicated temperatures, followed in 20 min by a second blanch at 93°C for 2 min to halt the firming process associated with heat activated pectin methylesterase. The blanched pods were then leached at 1°C overnight in 10 volumes distilled water to remove most of the pod salts. Pods were canned (225g pods per can), any indicated salt solutions, adjusted to pH 5.8 with the hydroxide of the same cation, were added, then distilled water was used to fill the #303 can. Filled cans were heated in a steam tunnel to 92°C, closed, and retorted at either 115°C or 100°C for the times indicated.

For the first experiment comparing the effects of cook time and blanch temperatures the work was replicated with three separate lots of beans and, in each lot, the cook treatments were conducted in duplicate. Various cook and soak treatments were carried out to give results used to calculate the salt cook effects. Beans were cooked in either distilled water or a calculated equilibrium concentration of 0.2M NaCl. While approximately 1 hr elapsed between filling and the start of retorting, salt equilibration between the fill solution and the pods cannot be considered complete before the retort step.

Salt soak treatments were carried out on pods that had been cooked in distilled water. These salt soaks consisted of soaking pods in solutions prepared by adding solid NaCl to the same liquor in which the pods had been cooked. The weight of NaCl added was calculated as the amount needed to obtain a 0.2M concentration after a calculated equilibration with the pods being returned to the salt augmented liquor.

The difference in firmness between pods cooked in salt solution and pods soaked in salt augmented liquor after cooking in distilled water, normalized with respect to firmness of pods cooked in distilled water, can be designated the salt cook softening effect. The salt cook softening effect has been calculated as the firmness of pods cooked in distilled water then soaked in salt augmented liquor minus the firmness of pods cooked in salt solution divided by the firmness of pods cooked in distilled water then multiplied by 100. The salt cook softening effect, as well as the salt cook pectin solubilizing effect described below, has no dimensions. They are measures that compensate for inherent differences between lots, are not sensitive to small variations in cooking time or temperature, and are adapted to comparisons between laboratories.

The salt cook pectin solubilizing effect was calculated as the salt cook liquor pectin minus the salt soak liquor pectin divided by the distilled water cook liquor pectin then multiplied by 100 (pectin was expressed as mg/ml). It was the additional pectin solubilized during the salt cook expressed as a per cent of the pectin solubilized during a corresponding distilled water cook.

All calculations of individual salt cook effects were done with beans of the same lot, blanch treatment, cook time, and cook temperature.

For the second experiment comparing the effects of KCl and $CaCl_2$ the work was replicated with two lots of beans and each lot had duplicated cook treatments. Cooking was for 20 min at 115°C. Some of the cooked beans were soaked in salt solutions as indicated, and these soaks were preceded by 2 days of leaching in 10 volumes distilled water at 1°C, with a change of water at the beginning of the second day. The purpose of the distilled water leach was to remove most of the salts that had been present in the cook media.

All salt solution soakings of cooked beans for both experiments were at 1°C for 3 or more days with occasional gentle stirring.

Firmness was measured as kg resistance to compression offered by 100g drained pods in a back extrusion test cell (Van Buren and Peck, 1981). Beans were warmed to near room temperature before firmness measurements. Pectin was determined by the modified m-hydroxydiphenyl method (Kintner and Van Buren, 1982). Statistical analysis was done using the MINITAB computer package (Ryan et al., 1980).

RESULTS & DISCUSSION

THE SALT COOK softening effect was obtained after different cooking times and temperatures using beans that had previously been blanched at 71°, 80°, and 90°C (Table 1). Longer cooking times at both cooking temperatures led to smaller salt cook softening effects. Decreases in the effects were also seen when higher blanch temperatures were used. The original firmness data (not given) showed lower values for kg resistance, when measured after cooking and after soaking in salt augmented liquor, as the cook times and blanch temperatures were increased.

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Table 1—Salt cook softening effect[a] due to inclusion of 0.2M NaCl in cook media

Cook conditions		Salt cook softening effect[b]		
Temp °C	Time minutes	Blanch temperature, °C 71	80	90
115	5	*	*	19 b
115	20	36 a	15 b	7 c
115	40	20 b	*	*
100	20	*	*	21 b
100	80	35 a	22 b	17 c
100	240	23 b	*	*

[a] Salt cook softening effect = [(salt soak firmness-salt cook firmness)/distilled water cook firmness)] × 100. Salt soaks in can liquor augmented to 0.2M NaCl were carried out on distilled water cooked beans from corresponding blanch treatments and cook times.

[b] Means in rows and columns at each cook temperature not followed by same letters were significantly different at the $P < 0.05$ level.

Table 2—Salt cook pectin solubilizing effect[a] due to inclusion of 0.2M of NaCl in cook media

Cook conditions		Salt cook pectin solubilizing effect[b]		
Temp °C	Time minutes	Blanch temperature, °C 71	80	90
115	5	*	*	55 b
115	20	156 a	20 c	10 c
115	40	77 b	*	*
100	20	*	*	106 b
100	80	254 a	28 c	47 c
100	240	86 b	*	*

[a] Salt cook pectin solubilizing effect = [(salt cook liquor pectin − salt soak liquor pectin)/distilled water cook liquor pectin)] × 100. Salt soaks in can liquor augmented to 0.2 M Na Cl were carried out on distilled water cooked beans from corresponding blanch treatments and cook times.

[b] Means in rows and columns at each cook temperature not followed by same letters were significantly different at the $P < 0.05$ level.

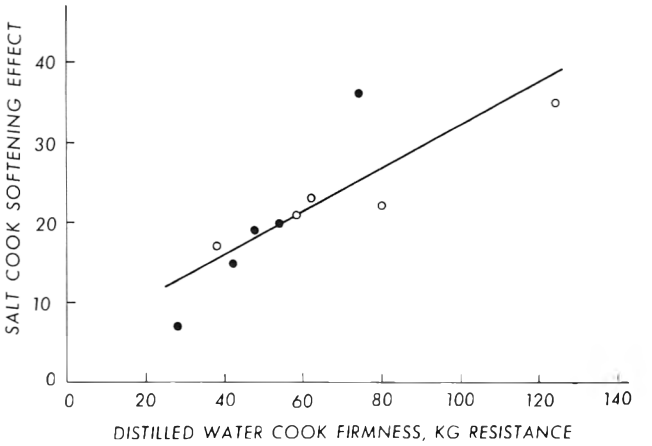

Fig. 1—Relation of salt cook softening effect to firmness of distilled water cook beans. Salt cook media was 0.2M NaCl. Cook temperatures were 100°C, (○), and 115°C, (●). The line has been drawn from the regression equation; Y = 5.2 + 0.27 X, R^2 = 0.71.

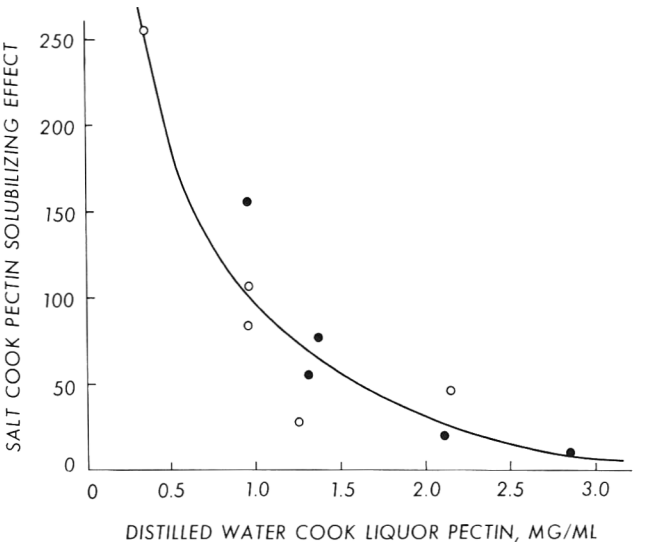

Fig. 2—Relation of salt cook pectin solubilizing effect to distilled water cook liquor pectin in canned snap beans. Salt cook media was 0.2M NaCl. Cook temperatures were 100°C, (○), and 115°C, (●). The line has been drawn from the regression equation: Y = − 120 + 216/ √X, R^2 = 0.87.

The salt cook softening effect can be compared with the firmness of beans of corresponding cook time and blanch temperature cooked in distilled water. As the distilled water cook firmness decreased so did the salt cook softening effect (Fig. 1). Under long cooking conditions giving the softest distilled water cook beans the difference between the salt soak firmness and the salt cook firmness became quite small. This indicates that the salt cook softening effect was taking place through components similar, if not identical, to those involved in the softening occurring during the distilled water cook, and that the presence of the salt accelerated changes in those components.

Pectins are solubilized during cooking, and the extent of solubilization at a given cooking time was increased by NaCl. The results given in Table 2 are expressed as the salt cook pectin solubilizing effect. Greater salt cook pectin solubilizing effects, at both cook temperatures, occurred with the lower blanch temperatures and the shorter cook times.

The magnitude of the salt cook pectin solubilizing effect was related to the concentration of liquor pectin found after the distilled water cook. There was a greater salt cook pectin solubilizing effect in those situations where the distilled water cook liquor pectins were lower (Fig. 2). Salt was particularly effective in accelerating solubilization during the early stages of cooking when relatively little pectin had been solubilized.

Numerous studies have established that decreases in firmness during cooking were accompanied by increase in the soluble pectin (Sistrunk and Cain, 1960; Sistrunk and Gonzalez, 1983). Some results for canned snap beans firmness and liquor pectin are given in Fig 3. The lower firmness values found for beans cooked in salt media can be ascribed to the Ca displacing action of the salt (Van Buren, 1984). Since it might be expected that there also would be a regular relation between the salt cook effects, salt cook softening effects were compared to the salt cook pectin solubilizing effects (Fig. 4). Both effects increased or decreased together. This suggests that the salt cook softening effect may have been largely the result of enhanced pectin solubilization. Such a solubilization could be due to β-eliminative depolymerization of pectins since this reaction in model systems is known to be accelerated by salts (Keijbets and Pilnik, 1974).

A general effect of salt was to lower the pH of the canned products compared to that seen for the corresponding distilled water cooks. This lowering was greatest at short cooking times and low blanching temperatures. There was little connection between the difference in pH and the salt cook softening effect. A graph comparing these two measurements (Fig. 5) showed considerable scattering of points, with a nonsignificant trend for less salt cook softening effect at smaller pH differences. Even more random scattering of points was seen when the pH differences were plotted against salt cook pectin solubilizing effect (not shown). It appears that the salt cook effects were separate from the pH lowering. The enhancement of softening by NaCl more than offset any inhibitory effect of a lower pH (Hughes et al., 1975).

Another method for determining the effects of salts was to cook the beans in selected salt solutions and then soak the cooked beans uniformly in a salt solution before measuring

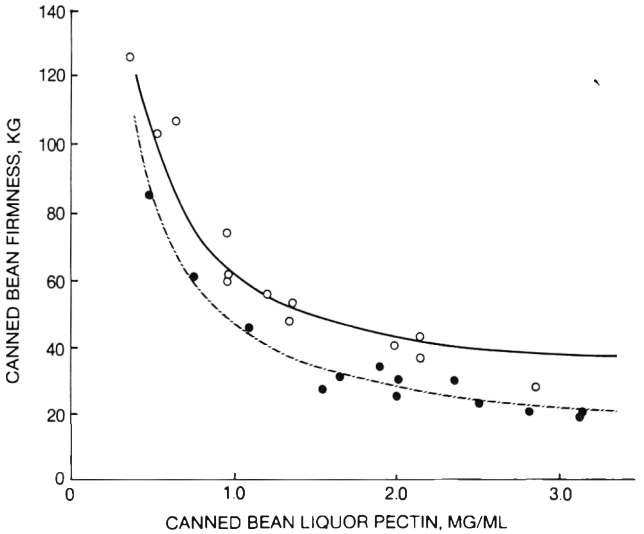

Fig. 3—Relation of canned bean firmness to canned bean liquor pectin. Plotted points, (○) for distilled water cook and (●) for 0.2M NaCl cook, are averages for three lots of beans treated at particular blanch temperatures, cook temperatures, and cook times. Line _____ is for distilled water cook and was drawn from the regression equation: $Y = 24 + 38/X$, $R^2 = 0.90$. Line — — — — — — is for 0.2M NaCl salt cook and was drawn from the regression equation: $Y = 9.8 + 37/X$, $R^2 = 0.97$.

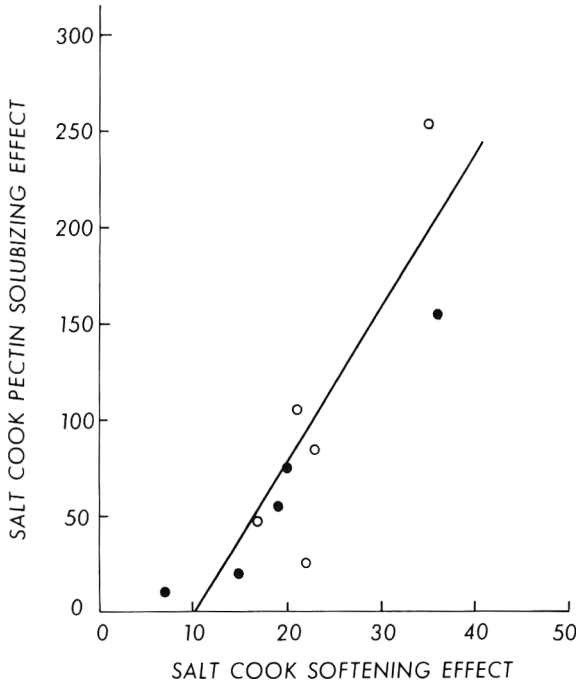

Fig. 4—Relation of salt cook pectin solubilizing effect to salt cook softening effect in canned snap beans. Salt cook media was 0.2M NaCL. Cook temperatures were 100°C, (○), and 115°C, (●). The line has been drawn from the regression equation: $Y = -76 + 7.45\ X$, $R^2 = 0.76$.

their firmness. The purpose of the uniform soak was to minimize the noncooking effects of the salts in the cook media (Van Buren, 1984). This was done in obtaining the data in Table 3 where the effects of $CaCl_2$ and KCl in the cooking media are presented.

Where firmness was measured on beans as they came from the can the $CaCl_2$ cook treatments were firmest and the KCl cook treatments were softest. However, after the cooked pods had been soaked in either 0.3M KCl-0.005M $CaCl_2$ or in 0.003M KCl-0.044M $CaCl_2$ it was found that the distilled water cook

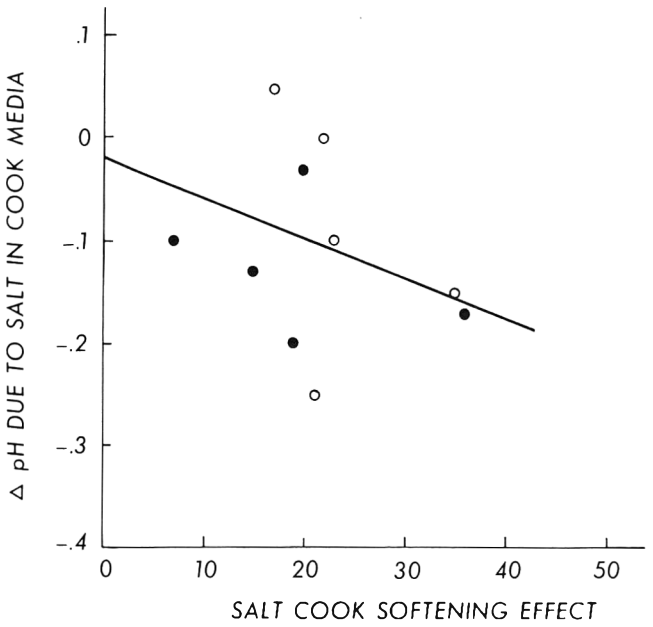

Fig. 5—Relation of the salt cook induced pH change to the salt cook softening effect in canned snap beans. Salt cook media was 0.2M NaCl, and the difference in pH was obtained as the difference between the pH of beans cooked in that media and beans cooked in distilled water. Cook temperatures were 100°C, (○), and 115°C, (●). The line has been drawn from the regression equation: $Y = -0.027 - 0.0037\ X$, $R^2 = 0.16$.

Table 3—Effect of KCl and $CaCl_2$ on firmness of canned beans

Cook media	Relative firmness[a] As canned	After KCl soak[b]	After $CaCl_2$ soak[c]	After HMP soak[d]
Distilled water	100 bc	111 ab	120 a	28 e
$CaCl_2$, 0.11 M	116 ab	96 c	108 b	28 e
KCl, 0.13 M	57 d	68 d	80 c	28 e

[a] Given as per cent firmness (kg resistance) of distilled water cook beans measured as they came from the can. Combined values for 71°C and 88°C blanched beans. Means in rows and columns not followed by same letters were significantly different at the $P < 0.05$ level.

[b,c,d] Canned beans soaked in 10 volumes of the indicated solutions for 3 days at 1°C: [b]KCl, 0.3M; $CaCl_2$, 0.005M soak solution; [c]$CaCl_2$, 0.044M; KCl, 0.003M soak solution; [d]Na hexametaphosphate, 0.002M soak solution.

beans were firmest. This indicates that the action of the $CaCl_2$ was complex, combining a firming action and a softening action, since both types of after-cook soaks reduced or eliminated differences in the firmness of the cooked beans due to differences in the concentration of Ca in the pods. KCl in the cook media had a softening effect similar to that seen earlier for NaCl. Very soft beans as well as elimination of firmness differences resulted from the soak in Na hexametaphosphate. This points to the Ca insolubilized pectinic acids as being the component of the pectin acted on by the salts to give the salt cook softening since Na hexametaphosphate is a well known extractant for this pectin fraction.

KCl in the cooking media caused an increase in the liquor pectin and a decrease in the pectin fraction insoluble in Na hexametaphosphate solution, Table 4. No such result was seen with $CaCl_2$, whose effect on softening was much less than that of KCl.

SUMMARY

THE PRESENCE of NaCl in the cooking media increased the rate of snap bean softening. This increase was greater for beans that had been blanched at a low temperature. The NaCl acted to reduce the differences in firmness of canned beans that had received different blanch treatments. KCl also increased the

Table 4—Effect of KCl and $CaCl_2$ on pectins of canned beans

	Pectin, mg/g wet weight[c]		
		Cooked pods	
Cook media	Can liquor	After $CaCl_2$ soak[a]	After HMP soak[b]
Distilled water	2.5 b	7.6 a	3.8 a
$CaCl_2$, 0.11M	2.6 b	7.3 a	3.7 a
KCl, 0.13M	3.5 a	6.1 a	2.8 b

[a,b] Canned beans soaked in 10 volumes of the indicated solutions for 3 days at 1 °C: [a]$CaCl_2$, 0.044M; KCl, 0.003M soak solution; [b]Na hexametaphosphate, 0.002M soak solution.

[c] Means in columns not followed by same letters were significantly different at the $P < 0.05$ level.

degree of softening. $CaCl_2$ was seen, after correction for its firming action, to cause a slight but significant softening during cooking. The softening action of the NaCl and KCl was accompanied by an increased rate of pectin solubilization.

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relaxation time selection involves, at least to some extent, an element of arbitrariness. Consequently, the model in its present form cannot be used to predict rheological behavior under stress conditions which are very different from those under which its constants were determined. It can be used, however, to assess rheological changes in food materials (e.g. as a result of processing, treatment or storage) by following the changes in its parameters as a function of time, temperature, etc.

Technical considerations

The method as described in this communication is based on the application of a microcomputer digitizer but requires a mainframe computer for the execution of the model program and the statistical calculations. This obviously complicates the technical procedure and necessitates rather elaborate and specific computer software. These complications, however, will almost certainly disappear when modern testing machines become more widely used in food research. Such machines are already designed to produce digital output and are or can easily be interfaced with a computer of appropriate capacity. It is, therefore, expected that once this modeling method is accepted, its application will be greatly facilitated by using these existing technical means.

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Bound Cations in Cucumber Pickle Mesocarp Tissue as Affected by Brining and $CaCl_2$

R.W. BUESCHER and J.M. HUDSON

ABSTRACT

The effects of brining, fermentation, storage, time of $CaCl_2$ addition and concentration of NaCl and $CaCl_2$ on Ca^{++}, Mg^{++}, Na^{+}, and K^{+} bound to middle lamella-cell wall material of cucumber pickle mesocarp tissue were examined. Changes in the amount of each bound cation occurred rapidly during the first 2 days of brining; levels then remained relatively stable during fermentation and storage if concentrations of NaCl or $CaCl_2$ in brines were not altered. NaCl reduced levels of bound Ca^{++}, Mg^{++}, and K^{+}, and the presence of $CaCl_2$ increased the amount of bound Ca^{++} by displacing the other cations. Delayed addition of $CaCl_2$ to brines enhanced the content of bound Ca^{++}, indicating that levels of bound Ca^{++} may not be related to maintenance of firmness.

INTRODUCTION

CALCIUM CHLORIDE is now commonly used in brines during cucumber (*Cucumis sativus L.*) fermentation and pickle storage. Its presence has been shown to protect against enzymatic and possible nonenzymatic softening (Buescher et al., 1979; 1981a,b; Buescher and Hudson, 1984; Hudson and Buescher, 1980; Fleming et al., 1978; Tang and McFeeters, 1983). Although a direct influence of Ca^{++} on enzymes responsible for softening has not been shown, it is known that Ca^{++} binds to polysaccharide polyelectrolytes especially galacturonans (Doesburg, 1965; Grant et al., 1973), resulting in a complex that hinders depolymerization by hydrolyases (Buescher and Hudson, 1984; Buescher et al., 1979). In contrast, the removal of Ca^{++} bound to middle lamella-cell wall (ML-CW) material enhances the rate and magnitude of galacturonan degradation by polygalacturonase (Buescher and Hobson, 1982).

In the absence of detectable softening enzyme activity, bound Ca^{++} probably protects against softening by preventing loosening and loss of cohesion of structure components rather than by retarding degradation (Ferguson, 1984). This seems to be accomplished in cucumber pickles and certain other fruits and vegetables by an alteration of galacturonan structure that is reflected by reduced solubility characteristics (Hudson and Buescher, 1985; Kaneko et al., 1984; Van Buren, 1979).

Ca^{++} bonding to galacturonans is influenced by several factors such as degree of esterification, ionic strength and pH (Kohn and Furda, 1967; Ferguson, 1984; Rendleman, 1978). During cucumber fermentation, galacturonans are rapidly deesterified (Hudson and Buescher, 1985; Tang and McFeeters, 1983); therefore the number of sites and density of consecutive sties should not limit the affinity for Ca^{++} (Grant et al., 1973; Kohn and Furda, 1967). Also, during fermentation, pH declines from about 5.0 to 3.5 or less and NaCl migrates into tissues until equilibrated at concentrations of 1–3M. This creates an acid-high ionic strength environment reported to be adverse to CA^{++} bonding (Ferguson, 1984; Kohn and Furda, 1967). Although bound Ca^{++} in relation to other cations is believed important for maintaining structural integrity, the effects of brining, fermentation and storage on bound cation levels in cucumber pickles have not been examined.

The objectives of this contribution were to determine the effects of fermentation, storage in brine and concentrations of $CaCl_2$ and NaCl on levels of bound Ca^{++}, Mg^{++}, Na^{+}, and K^{+} in ML-CW material from cucumber pickle mesocarp tissue. In addition, the effects of delayed $CaCl_2$ treatment of brines containing pickles on these bound cations were determined.

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MATERIALS & METHODS

Source, fermentation and storage

Fresh size no. 3 (3.8 – 5.1 cm diameter) cucumbers (Carolina var.) were obtained from Atkins Pickle Company (Atkins, AR). At the time of arrival from the field, fruits were sorted for uniformity of size, shape and freedom from defects and packed tightly in 3.8L (1 gal) glass jars that contained 1.5L brine to provide pack-out yields of 60% cucumbers and 40% liquid. Brines containing 4.4% NaCl were prepared by adjusting the pH to 3.0 with acetic acid (0.08%) adding K-sorbate (0.1%) and adjusting the pH to 5.0 with 10N NaOH (Hudson and Buescher, 1985). Anhydrous $CaCl_2$ (Peladow, Dow Chemical Co.) was added to brines of designated treatments. The brines containing submerged cucumbers were inoculated with *Pediococcus cerevisiae* (Microlife Technics, Aferm 772) and held at 22 ± 2°C during fermentation and storage. After fermentation was completed the containers were loosely capped to reduce evaporation.

$CaCl_2$, fermentation and storage

Changes in bound cations of cucumbers packed in brines containing initial levels of 0 (control) or 100 mM $CaCl_2$ (wt/liquid volume basis) were determined during fermentation and storage. Samples were taken at 0 (not brined), 2, 5, 10, 16, 29, and 60 days after brining.

Delayed $CaCl_2$ treatment

To determine the effects of delayed $CaCl_2$ treatment on levels of bound cations, $CaCl_2$ was added to provide a concentration of 100 mM in the free liquid portion of the brines at 0 (initial), 1, 4 or 8 wk after brining. Samples without the addition of $CaCl_2$ served as the controls. Analyses of all treatments were conducted 4 months after brining.

$CaCl_2$ and NaCl concentrations

The effects of $CaCl_2$ and NaCl concentration on bound cations were examined by fermenting cucumbers in brines initially containing 4.4% NaCl and 0, 33, 66, or 99 mM $CaCl_2$. After fermentation was completed (2wk), NaCl was added to each of the $CaCl_2$ treatments to provide equilibrated concentrations of 2.5, 5.0, 10.0 or 15.0% based on the total container volume. Samples were assayed 9 months after brining.

Extraction and analyses

Mesocarp tissues dissected from 10 fruits of each treatment were blended in four volumes (v/wt) of 95% ethanol and vacuum filtered through Whatman no. 4 ashless filter paper. The residue was twice suspended in 95% ethanol, blended, filtered and dried in a vacuum oven at 50°C, ground to a fine powder (80 mesh screen) and stored desiccated.

The middle lamella-cell wall (ML-CW) material was weighed (50 mg/replication) into borosilicate digestion tubes and wet ashed with

concentrated H_2SO_4 (3 mL) for 30 min followed by the addition of 30% H_2O_2 (0.5 mL) and heating at 280 ± 20°C for 1 hr in a digestion block. If solutions were not clear, a few drops of H_2O_2 were added and heat was reapplied for 15 min. When cooled, solutions were diluted, washed through Whatman no. 541 ashless filter paper and adjusted to 50 mL with deionized water. Glassware used for extractions was acid-washed to avoid contamination, and appropriate blanks without tissue extracts were included to correct for levels of cations in the solvents. Concentrations of Ca^{++}, Mg^{++}, Na^{+}, and K^{+} were determined by atomic absorption spectrophotometry in the Water Testing Laboratory, Dept. of Agronomy, Univ. of Arkansas.

Fermentation and storage brines were assayed for pH, titratable acidity, NaCl and polygalacturonase activity as previously described (Buescher et al., 1981a; Hudson and Buescher, 1985). Samples of brines were also assayed for Ca^{++} by atomic absorption spectrophotometry.

Replication and statistical analysis

Fermentation and brine storage treatments were duplicated. Three replications from each treatment were prepared for bound cation analysis. Data for bound cation levels were statistically examined by analysis of variance ($P \leq 0.01$), and least significant differences were calculated for mean separation (Steele and Torrie, 1960).

RESULTS

Brine and cucumber characteristics

Fermentation and cucumber pickle characteristics were normal in all containers. No yeast or mold growth was observed, and polygalacturonase activity was negligible. Brines had titratable acidity of 1.3 ± 0.2% as lactic acid and pH levels of 3.4 ± 0.1. Once established the brine characteristics remained constant during storage.

Fermentation and storage

Most changes in Ca^{++}, Mg^{++}, Na^{+}, and K^{+} bound to ML-CW material from cucumber mesocarp tissue occurred within the first 2 days of brining (Table 1). Once established the levels of bound cations remained relatively constant regardless of fermentation activity and storage duration. Exposing cucumbers to the control brine treatment reduced the amount of bound Ca^{++}, Mg^{++}, and K^{+} while bound Na^{+} was increased. Including $CaCl_2$ in the brine further reduced the levels of Mg^{++} and K^{+}, attenuated the increase in Na^{+} and substantially enhanced the amount of Ca^{++}. The presence of $CaCl_2$ increased the total microequivalents of bound cations.

Delayed $CaCl_2$ treatment

Similar to the results in the previous experiment, the presence of $CaCl_2$ in fermentation and storage brines increased the amount of bound Ca^{++} and reduced levels of bound Mg^{++}, Na^{+} and K^{+} (Table 2). When the addition of $CaCl_2$ was delayed, more Ca^{++} was associated with the ML-CW material while levels of Mg^{++}, Na^{+} and K^{+} apparently were not affected. The amount of bound Ca^{++} was enhanced to levels greater than the amount of Mg^{++}, Na^{+} and K^{+} that was displaced, causing an increase in the total bound microequivalents.

$CaCl_2$ and NaCl concentrations

Increased concentrations of NaCl tended to reduce the amount of bound Ca^{++}, Mg^{++} and K^{+} and increase levels of bound Na^{+} (Table 3). The maximum influence of NaCl on bound Ca^{++}, Mg^{++}, and Na^{+} was usually attained by 10% NaCl with a further increase of NaCl to 15% having little or no affect. However, with few exceptions, bound K^{+} was significantly reduced by each increase in NaCl concentration. Elevating the level of $CaCl_2$ in brines increased bound Ca^{++} and reduced bound Mg^{++}, Na^{+} and K^{+}. These effects of $CaCl_2$ were largest in 2.5% NaCl brines and diminished as the NaCl content of brines was increased. Differences in bound Ca^{++} were not significant between treatments of 30 and 47 mM $CaCl_2$ when combined with 15% NaCl; however, the amount of bound Ca^{++} in these treatments was significantly more than the amount bound to ML-CW material when $CaCl_2$ was not added to the brines containing 15% NaCl.

As observed in the other experiments, NaCl and $CaCl_2$ had opposing effects on the total bound microequivalents determined from summation of cationic charges.

DISCUSSION

RATHER THAN BEING a gradual process, the migration of Na^{+} and Ca^{++} into cucumber mesocarp tissue to concomitantly bond to ML-CW material occurred very rapidly. Within two days from initiating brining, most changes in bound cations were completed, even though fermentation and accompanying changes in lactic acid production and pH continued for about 10 days. Changes in characteristics of galacturonans during brining of cucumbers have been shown to occur rapidly with the rate of demethylation favorable for rapid bonding of cations (Hudson and Buescher, 1985). Kaneko et al. (1984) found similar results when changes in bound cations were measured 4 and 14 days after brining various Chinese vegetables. The most important influence regulating levels of bound cations was the concentration of NaCl and/or $CaCl_2$. New levels were established whenever the concentration of these salts in brines were adjusted. This could have practical importance, since NaCl concentrations are usually increased after fermentation for pickle storage. The pickles are then de-salted and placed in new brine during processing. Although the effects of processing procedures on bound cations are unknown, major changes should occur when NaCl concentrations are elevated after fermentation.

Individually, Na^{+} and Ca^{++} were effective in displacing each other as well as Mg^{++} and K^{+}. To obtain similar responses the level of NaCl had to be several times greater than the concentration of $CaCl_2$ which corresponds with the binding

Table 1—Levels of cations bound to middle lamella-cell wall material of cucumber mesocarp tissue as affected by brining, fermentation, storage and $CaCl_2$

	Bound cations (micromoles/g)									
	Ca^{++}		Mg^{++}		Na^{+}		K^{+}		Total microeq/g[b]	
Days in brine	Treatment[a]									
	Control	$CaCl_2$	Control	$CaCl_2$	Control	$CaCl_2$	Control	$CaCl_2$	Control	$CaCl_2$
0	202	202	91	91	43	43	325	325	954	954
2	147	499	53	29	291	143	161	61	852	1260
5	135	531	37	12	274	157	130	54	748	1297
10	125	546	25	12	291	191	146	69	737	1376
16	147	536	29	12	291	165	128	51	771	1312
29	147	554	25	12	283	152	123	46	750	1330
60	150	569	29	12	300	165	138	54	796	1381
LSD 0.01	47		8		43		36		189	

[a] Cucumbers were submerged in brines containing 4.4% (750 mM) NaCl and 0 (control) or 100 mM $CaCl_2$ which equilibrated to 2.4% (411 mM) NaCl and 5 ± 2 (control) or 45 ± 5 mM $CaCl_2$.

[b] Total microequivalents/g is the sum of net positive charges of the cations.

Table 2—Effect of delayed addition of $CaCl_2$ to brines on levels of cations bound to middle lamella-cell wall material of cucumber mesocarp tissue

Time of $CaCl_2$ addition (wk after brining)[a]	Bound cations (micromoles/g)				
	Ca^{++}	Mg^{++}	Na^{+}	K^{+}	Total microeq/g[b]
Control (no $CaCl_2$)	132	37	274	194	806
0 (no delay)	474	16	161	79	1220
1	551	16	139	74	1347
4	546	16	157	77	1358
8	579	19	152	77	1425
LSD 0.01	37	8	23	46	159

[a] Cucumbers were submerged in brines containing 4.4% NaCl and 0 (control) or 100 mM $CaCl_2$ (no delay). For delay treatments, $CaCl_2$ (100 mM) was added to brine at 1, 4 or 8 wk after brining. Levels of NaCl and $CaCl_2$ equilibrated to 2.4 ± 0.2% and 43 ± 4 mM, respectively. Cucumber tissues were extracted 4 months after brining.
[b] Total microequivalents/g is the sum of net positive charges of the cations.

Table 3—Effect of NaCl and $CaCl_2$ concentrations on cations bound to middle lamella-cell wall material of cucumber mesocarp tissue[a]

$CaCl_2$ added to brine (mM)	Equilibrated $CaCl_2$ (mM)	NaCl (%)			
		2.5	5.0	10.0	15.0
		Bound Ca^{++}[b]			
0	5 ± 2	150f	117gh	82i	85hi
33	19 ± 3	244c	200de	155f	150f
66	30 ± 5	319b	249c	197de	182ef
99	47 ± 7	399a	294b	229cd	195e
		Bound Mg^{++}[b]			
0	5 ± 2	27a	26ab	18cd	16cde
33	19 ± 3	25ab	21bc	15de	13defg
66	30 ± 5	21bc	15de	15de	11efg
99	47 ± 7	16cde	8g	14def	9fg
		Bound Na^{+}[b]			
0	5 ± 2	265de	313abc	348a	343a
33	19 ± 3	217f	287cd	335ab	330ab
66	30 ± 5	193f	261de	290cd	313abc
99	47 ± 7	145g	230ef	275cd	304bc
		Bound K^{+}[b]			
0	5 ± 3	125a	94b	68de	54g
33	19 ± 3	99a	84c	68de	51g
66	30 ± 5	85c	74d	57fg	37h
99	47 ± 7	62ef	65e	51g	43h
		Total microequivalents/g[b]			
0	5 ± 2	744def	693fgh	616gh	599h
33	19 ± 3	854bcde	813cdef	743def	707fgh
66	30 ± 5	958ab	863bcd	771def	736efg
99	47 ± 7	1037a	899bc	812cdef	755def

[a] Cucumbers were placed in brines containing 4.4% NaCl and 0, 33, 66, or 99 mM $CaCl_2$ which equilibrated to 2.5% NaCl and 5, 19, 30 or 47 mM $CaCl_2$. After 2 wk each of the $CaCl_2$ treatments were adjusted to contain equilibrated concentrations of 2.5, 5.0, 10.0, and 15.0% NaCl. Tissues were extracted 9 months after brining.
[b] Levels of bound cations are expressed as micromoles/g of dried middle lamella-cell wall material. Mean separation of bound cations based on least significant difference, 1% level. Values within each section followed by the same letter are not significantly different.

sites having a greater affinity for Ca^{++} than Na^{+} (Rendleman, 1978). In combination, Na^{+} and Ca^{++} acted cooperatively in displacing Mg^{++} and K^{+} while being competitive between each other for bonding sites. Regardless of NaCl concentration (427–2567 mM) the presence of 30 or 47 mM $CaCl_2$ more than doubled the amount of bound Ca^{++}, indicating that the Ca^{++} had the competitive advantage.

Brining in the absence of $CaCl_2$ consistently reduced the total microequivalents of bound cations. The results of Kaneko et al. (1984), when calculated as total microequivalents, also showed that brining caused a similar reduction. These effects of brining were surprising, since the amount of galacturonan is not altered by brining, and demethylation is greatest when $CaCl_2$ is absent (Hudson and Buescher, 1985; Tang and McFeeters, 1983). Possibly cations not measured in this study contributed to the apparent reduction in microequivalents. In contrast, the presence of Ca^{++} increased the total microequivalents of bound cations which may be accounted for by the increased number of bonding sites, the affinity of galacturonans for Ca^{++} and possible association of Ca^{++} with other polysaccharides (Rendleman, 1978).

Assuming that the level of bound Ca^{++} is directly related to maintaining firmness, then delaying addition of $CaCl_2$ to brines or fermentation and storing in low (2.5%) NaCl concentration with $CaCl_2$ should be most effective. This is partially true in the latter case since maximum firmness is attained with low NaCl levels and $CaCl_2$. However, higher levels of NaCl along with $CaCl_2$ result in similar levels of firmness (Hudson and Buescher, 1985), even though the amount of bound Ca^{++} is reduced with high NaCl concentrations. The relationship of bound Ca^{++} to firmness is even more erratic when the effects of delayed $CaCl_2$ addition are considered, since delayed treatments fail to maintain firmness (Buescher et al., 1981a; Tang and McFeeters, 1938) but result in greater levels of bound Ca^{++} (Table 2). While the effects of Ca^{++} on firmness have been established, the mechanism of firming does not appear to be completely based on the amount of Ca^{++} bound to cell structural material.

In summary, levels of specific cations bound to ML-CW material of cucumber pickle mesocarp tissue were dependent on the concentration of NaCl and/or $CaCl_2$ in the brining solutions and they were independent of fermentation and storage duration. Brines containing low NaCl concentrations allowed for greater amounts of Ca^{++} to be bound. Although the quantity of bound Ca^{++} declined with increasing NaCl concentrations, it was evident that the affinity of ML-CW material was greater for Ca^{++} than for Na^{+}.

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Relationship Between Degree of Pectin Methylation and Tissue Firmness of Cucumber Pickles

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ABSTRACT

The relationship between pectin methylation and tissue firmness was examined in cucumber pickles exposed to pre-brining and brining treatments. Tissue treated with $CaCl_2$ prior to or during brining, blanched before brining or held at 2°C during brine storage resisted softening. Although all treatments reduced the degree of esterification (DE) of pectic substances, less demethylation occurred in treatments that protected against softening. Join point regression analysis of the data indicated that maximum firmness of mesocarp tissue was attained when the DE of pectins was 12.3 ± 1.2 or greater. Firmness declined concomitantly when the DE declined below 12.3 ± 1.2. Methods that protect against excessive demethylation of pectins appear to be important in retarding softening of cucumber pickle tissue during storage in brine.

INTRODUCTION

CUCUMBER (*Cucumis sativus*) pickles tend to soften during brine storage, especially in low NaCl conditions (Tang and McFeeters, 1983). Dissolution of cell wall components, particularly the pectic substances, has been related to softening of pickle tissue. Ca^{++} and/or high NaCl levels prevent both enzymatic and nonenzymatic softening of brined cucumbers (Buescher and Hudson, 1984; Buescher et al., 1979; Hudson and Buescher, 1980). Ca^{++} also can increase tissue firmness, and its efficacy is greatest when it is added initially to brining solutions (Buescher et al., 1981; Tang and McFeeters, 1983).

Firming and resistance to softening induced by Ca^{++} have been attributed to the formation of Ca-pectates that increase middle lamella-cell wall rigidity (Grant et al., 1973) and resistance to degradation by polygalacturonase (Bateman, 1964). Demethylation of pectins, primarily by pectinmethylesterase (PME), has been proposed as a mechanism for tissue firming by creating more free carboxyl groups for Ca^{++} binding (Bartolome and Hoff, 1972). Pectic substances of fresh cucumbers are from 35–50% de-esterified (Fukushima, 1978; Hudson and Buescher, 1985), which should allow abundant sites for Ca^{++} binding, although relationships between degree of esterification (DE) and Ca^{++} induced firmness have not been clearly defined.

Independent of the formation of Ca-pectates, the DE has been shown to play an important role in the rigidity and configuration of pectic substances whereby the stiffness of the pectic macromolecule increases with increasing methoxyl content (Michel et al., 1982). Leeper and Dull (1972) demonstrated that demethylation of pectin results in a transition from an ordered coiled molecular structure to a linear elementary fibril configuration. This ordered configuration is responsible for the ability of high methoxyl pectins to form a gel with the addition of sugar and acid, while low methoxyl pectins require the presence of multivalent cations to gel (Dull and Leeper, 1975).

From previous studies with cucumber pickle tissue, Ca^{++} protected against softening by reducing the solubilization and deesterification of pectic substances (Hudson and Buescher, 1985). Thus, firm tissue seems to be associated with the formation of insoluble and methylated pectic substances. This study was undertaken to test the hypothesis that firmness of cucumber tissue is associated with the degree of esterification of cucumber pectins. Our approach to this premise was to treat cucumbers by various methods to produce tissue with a range in the DE of pectins and then to analyze the relationship between tissue firmness and the DE by regression analysis.

Since demethylation probably occurs as a result of cucumber pectin methyl esterase (PME) (Bell et al., 1951; McFeeters et al., 1985), treatments were selected for their ability to retard demethylation (e.g., ionic species, temperature extremes, low pH). Ca^{++} and Na^{+} have been shown to inhibit PME activity (Pressey and Avants, 1972; Van Buren et al., 1962). Therefore, $CaCl_2$ and/or NaCl were employed in brining and/or infiltration treatments to retard demethylation. To check the possibility of end product inhibition from demethylation of pectins by PME, the effects of infiltrating methanol were observed. The effects of temperature on tissue firmness and the DE of pectins were evaluated by either blanching tissues prior to brining or using 2°C or 24°C storage. Activation of PME by blanching at 50–70°C could result in lower levels of methylated pectins (Bartolome and Hoff, 1972), while using low temperature storage might reduce PME activity and result in maintenance of a high level of methylated pectins. The optimum pH for activity of PME from most plants is in the alkaline range (pH 7.5–8.0) (Bell et al., 1951; Pressey and Avants, 1972; Van Buren et al., 1962). Thus, low pH inactivation of PME by using acid brine would be expected to result in higher levels of methylated pectins in brined tissues.

Two separate experiments were conducted. In Experiment I the effects of fermentation, acid storage, $CaCl_2$, blanching and infiltration treatments on the firmness and pectin esterification of cucumber tissues were determined. Experiment II was designed to evaluate the effects of $CaCl_2$, brine infiltration and storage temperature on the firmness and pectin methylation of tissues stored in acid brine without fermentation.

MATERIALS & METHODS

Source of cucumbers

Fresh pickling cucumbers ('Carolina') were obtained from a local wholesaler. Size No. 3 (3.8–5.1 cm diameter) fruit, free from decay and mechanical damage, were used after washing. Cucumbers were sliced to a length of 3.8 cm for use in two separate experiments; end pieces were discarded.

Experiment I

Prior to brining, random samples of fresh cucumber tissues were either blanched for 3 min in boiling water (to an internal temperature of 45–50°C) and then cooled in tap water for 3 min or vacuum infiltrated in solutions containing 4.4% $CaCl_2$, 15.0% NaCl, 4.4% $CaCl_2$ and 15.0% NaCl or 3.9% CH_3OH. Vacuum infiltration of treatment solutions was accomplished by holding tissue submerged for 5 min at ca 150mm Hg and then slowly releasing the vacuum.

Fresh, blanched or infiltrated cucumber tissues were packed in 3.8L glass jars containing brine that equilibrated at 2.4% NaCl and 0.04%

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K-sorbate, except for the two NaCl infiltration treatments which had a final concentration of 3.0% NACl. Other samples of fresh tissues and tissues infiltrated in the two $CaCl_2$ solutions were placed in similar brines that contained $CaCl_2$ to provide an equilibrated level of 0.4%. Brines were buffered by first adjusting the pH to 3.0 with glacial acetic acid and then to pH 4.8 with NaOH. The cucumber-to-brine ratio was approximately 60:40 (w/v). The brined cucumbers were inoculated with ca 10^8 cells of *Pediococcus ceresisiae* (Aferm 772, furnished by Microlife Technics, Inc., Sarasota, FL) per 3.8 L. After fermentation, brine pH and titratable acidity (TA) for all treatments averaged 3.36 and 0.10 meq/mL.

To determine the effect of storage at low pH without fermentation on firmness and methoxyl content, two additional groups of cucumbers were packed in jars containing brines that equilibrated at pH 3.36, 0.228 meq/mL TA (acetic acid) and 2.0% NaCl with or without 0.3% $CaCl_2$. These acidified storage treatments did not contain K-sorbate. Fermentation and/or storage of treatments in Experiment I were conducted in a thermostatically controlled room held at 24°C.

Experiment II

Another experiment was designed to evaluate the effects of brine infiltration, $CaCl_2$ and storage temperature on firmness and pectin methylation of cucumber tissues stored in acidified brines (without fermentation). Two samples of fresh cucumber tissues were placed in brines that equilibrated at pH 3.45, 0.242 meq/mL TA (acetic acid) and 2.3% NaCl with or without 0.3% $CaCl_2$. Temperatures of brine and cucumber tissue were equilibrated to 24°C prior to brining. Two other groups of tissues were infiltrated for 5 min at ca 150 mm Hg in the brines described above (at 24°C) and then stored in the infiltration brines. Also, two groups of tissues were packed in the brines as described above except that the cucumbers and brines were equilibrated to 2°C prior to packing. Each set of brined cucumbers was subsequently stored at the temperature at which they were equilibrated prior to brining.

Extractions and analyses

Analyses for both experiments were conducted 105 days after brining. TA, pH and firmness were determined as previously described (Buescher et al., 1981). The amount of chloride was measured to estimate NaCl concentrations by titrating a 1.0 mL sample of brine in 10 mL deionized H_2O, 4 drops 2/3N H_2SO_4 and 2 drops diphenylcarbazone indicator (5 mg/2 mL methanol) with 0.1N $Hg(NO_3)_2$ (modified from Sigma Technical Bulletin No. 830, Sigma Chemical Company, St. Louis, MO). Fresh cucumbers, blended and filtered through Miracloth, had a pH of 5.63, TA of 0.013 meq/mL and NaCl level of 0.16%.

Mesocarp tissue was dissected from 10 fruits, peel removed and discarded, blended with at least four volumes (wt/vol) of 95% ethanol and then filtered through Whatman No. 40 ashless filter paper in a Buchner funnel. The residue was twice resuspended in 95% ethanol, blended and filtered. The alcohol insoluble solids (AIS) were collected and dried under vacuum at 50°C.

Total pectins were determined by mixing 7.5 mg AIS with 5.0 mL of cold concentrated H_2SO_4 in tubes held in an ice-water bath until the AIS were well suspended (about 5 min) (Ahmed and Labavitch, 1977). Then 1.25 mL of deionized H_2O was added dropwise as the mixture was stirred. After 5 min another 1.25 mL of deionized H_2O was added dropwise. The AIS were digested for 1 hr in an ice-water bath, and aliquots were assayed for uronic acids using the m-hydroxydiphenyl method as described by Kintner and Van Buren (1982).

Methoxyl content was measured by hydrating 25 mg of AIS with 16 mL deionized H_2O, saponifying in stoppered tubes with 4 mL of 5N NaOH for 30 min at room temperature, cooling in an ice-water bath and acidifying with 5 mL of cold 9N H_2SO_4. Samples (1.0 mL) were transferred to stoppered test tubes held in an ice-water bath and assayed as described by Wood and Siddiqui (1971) with the exceptions that stoppered tubes were always used and color development was conducted at room temperature (22-24°C) for 60 ± 1 min. Percent methoxyl values were converted to degree of esterification (DE) using the theoretical maximum of 16.32% of methoxyl content being equivalent to a DE of 100 (Doesburg, 1965).

Firmness was measured by a U.C. Fruit Firmness Tester (Western Industrial Supply, Inc. San Francisco, CA) equipped with an Ametek LKG-14 gauge and a 5/16-inch (0.79 cm) tip. Firmness measurements for all treatments were made both perpendicularly through the peel and mesocarp and longitudinally through the mesocarp tissue.

Statistics

Duplicate samples were used for all brining treatments. Firmness measurements of each treatment were made on 20 cucumber pieces. Total pectins and methoxyl content were assayed from four samples using repetitions from each duplicate treatment. Statistical analysis for least significant differences and correlation coefficients were calculated as described by Steel and Torrie (1960). Regression analysis of tissue firmness and the degree of pectin esterification was accomplished by using join point analysis as described by Hudson (1966).

RESULTS & DISCUSSION

Experiment I

The pre-brining and brining treatments caused considerable variation in cucumber tissue firmness and in the degree of esterification (DE) of pectins in mesocarp tissue (Table 1). Firmness measurements through the mesocarp and peel tissues were similarly affected by the treatments.

Fresh cucumber tissues were firm and had the greatest DE of pectins. Treatments with $CaCl_2$ or blanching protected against softening during storage in brine. All brining treatments reduced the DE of pectins; however, blanching prior to fermentation or the presence of $CaCl_2$ in brines retarded the extent of demethylation. Vacuum infiltration of $CaCl_2$ prior to brining tended to be less effective than brining in solutions containing $CaCl_2$ in retarding tissue softening and demethylation of pectins. The greatest softening and reduction in DE occurred by the fermentation control, vacuum infiltration of NaCl and CH_3OH treatments. Although the intention of infiltrating NaCl into cucumber tissue prior to brining was to reduce the DE of pectins, storage in brines accomplished similar results. Infiltration of methanol was anticipated to inhibit demethylation by product inhibition of PME; however, it had a stimulatory action on softening and demethylation. Storage in acid brine without fermentation reduced the DE to levels similar to those observed in pectins from the fermentation control treatment, although tissue softening was reduced by storage in the acid brine treatment.

Experiment II

A second experiment with cucumbers stored in acid brine solutions without fermentation confirmed that $CaCl_2$ protected tissues from softening and reduced the amount of demethylation of pectins (Table 2). Except for causing a rapid cured appearance of the pickle tissue, vacuum infiltration of brines had similar effects on firmness and DE as did the treatments conducted at 24°C without vacuum infiltration. Storage at 2°C in the absence of $CaCl_2$ was as effective in maintaining firmness and reducing demethylation as storage at 24°C in brines containing $CaCl_2$. The highest firmness and DE values were obtained from tissues stored at 2°C in brines containing $CaCl_2$.

Tissue firmness related to the DE of pectins

The relationship between tissue firmness and the DE of pectins from cucumber pickles is shown in Fig. 1. The data presented in Fig. 1 are the combination of results from experiments I and II. Join point regression analysis indicated that the data were best described by two lines ($p<0.001$). The two lines intersected at a DE of 12.3 ± 1.2. Firmness was directly related to the DE of pectins when the DE was less than 12.3. However, when the DE values were greater than 12.3, tissue firmness was unaffected by the level of methylated pectins since it was maintained at about 5.06 kg. Large changes in firmness resulted from relatively small changes in the DE of pectins when the DE was less than 12.3.

Changes in the methyl ester content of pectins have been demonstrated to cause conformational changes in the pectin macromolecule, especially when the DE is from 0–15 (Dull and Leeper, 1975; Leeper and Dull, 1972). Demethylation caused pectins to uncoil from their rigid, native, molecular

Table 1—Firmness and pectin esterification (DE) as influenced by pre-brining and brining treatments of cucumber tissue[a]

Pre-brining treatment	Brining treatment[b]	Firmness[c] (kg) Mesocarp	Firmness[c] (kg) Peel	DE
None	None (fresh tissue)	5.3ab	9.5abc	43.3a
None	Acid, no fermentation	4.6 cd	7.2c	8.1f
None	Acid + $CaCl_2$, no fermentation	5.1abc	9.3ab	14.7c
None	Fermentation control	2.8e	5.8d	7.5f
None	Fermentation + $CaCl_2$	4.7 bcd	9.2ab	13.6c
Blanched (3 min boiling H_2O)	Fermentation	4.7 bcd	8.7b	18.5b
$CaCl_2$ (4.4%) infiltrated[d]	Fermentation	4.4d	8.6b	9.5e
$CaCl_2$ (4.4%) infiltrated	Fermentation + $CaCl_2$	5.4a	10.0a	14.3c
NaCl (15.0%) infiltrated	Fermentation	2.8e	5.2de	6.9fg
NaCl (15.0%) + $CaCl_2$ (4.4%) infiltrated	Fermentation	4.6cd	8.6b	10.8d
CH_2OH (3.9%) infiltrated	Fermentation	2.9e	4.6e	6.0g

[a] Treatments and storage conducted at 24°C. Analyses were conducted 105 days after brining. Mean separation by $LSD_{.01}$ test; values within columns with same letter are not significantly different. Coefficients of variation for mesocarp firmness, peel firmness and DE were 16.0%, 13.6% and 4.7%, respectively.

[b] Fresh cucumber tissues were placed in brines which equilibrated at pH 3.36, 0.100 meq/ml titratable acidity and 2.4% NaCl with or wihtout 0.4% $CaCl_2$, except for the acid treatments that equilibrated at 0.228 meq/mL titratable acidity (acetic acid) and 2.0% NaCl with or without 0.3% $CaCl_2$ and the two NaCl infiltration treatments that equilibrated at 3.0% NaCl.

[c] Firmness was measured longitudinally through the mesocarp tissue and perpendicularly through the peel.

[d] Fresh cucumber slices were infiltrated for 5 min at 150 mm Hg in pre-brining solutions prior to placing in the designated brining treatment.

Table 2—Firmness and pectin esterification (DE) of cucumber tissue stored in acid brine without fermentation as affected by $CaCl_2$, brine infiltration and storage temperature[a]

$CaCl_2$ treatment of brines[b]	Storage temperature (°C)	Firmness[c] (kg) Mesocarp	Firmness[c] (kg) Peel	DE
No treatment (fresh tissue)		5.3ab	9.5abd	48.8a
−	24	4.0c	6.4d	9.6d
+	24	5.3ab	8.7bc	15.8c
−	2	4.9b	9.2bc	16.3c
+	2	5.6a	10.2a	18.7b
− Brine infiltrated	24	4.0c	6.5d	10.6d
+ Brine infiltrated	24	5.0ab	8.4c	15.8c

[a] Analyses were conducted 105 days after brining. Mean separation by $LSD_{.01}$ test; values within columns with same letter are not significantly different. Coefficients of variation for mesocarp firmness, peel firmness and DE were 18.1%, 11.5% and 3.8%, respectively.

[b] Fresh cucumber tissues were placed in brines that equilibrated at pH 3.45, 0.242 meq/mL titratable acidity (acetic acid) and 2.3% NaCl with (+) or without (−) 0.3% $CaCl_2$. Infiltrated treatments consisted of fresh cucumber tissues vacuum infiltrated for 5 min at 150 mm Hg in the designated brining treatment and subsequently stored in the same brine.

[c] Firmness was measured longitudinally through the mesocarp and perpendicularly through the peel.

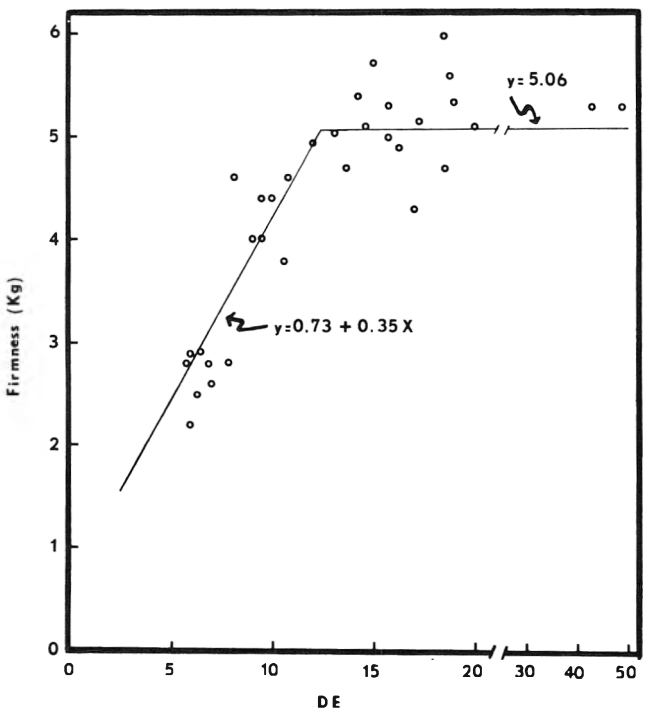

Fig. 1—Relationship between firmness of cucumber mesocarp tissue and the degree of esterification (DE) of pectins.

configuration to form an elastic, linear configuration. Also, Michel et al. (1982) concluded that the stiffness of pectin macromolecules declines as the DE decreases. Although these relationships between pectin configuration, DE and rigidity were based on observations of purified pectins, we believe that they provide a basis for explaining the relationship found between the firmness of cucumber tissues and the DE of pectins.

McFeeters et al. (1985) were unable to observed a direct relationship between the DE of pectins and firmness of pickle slices from their blanching, $CaCl_2$ and storage in acid brine treatments, although blanching and $CaCl_2$ were reported to reduce the amount of pectin demethylation. However, their observations do not conflict with our results, since few of their treatments allowed the DE of pectins to decline below the critical level of of 12.3 ± 1.2.

Previous studies have indicated that Ca^{++} is most effective in retarding cucumber pickle softening when it is present in the initial brining solutions, rather than delaying its addition (Buescher et al., 1981; Tang and McFeeters, 1983). Although delaying the addition of Ca^{++} increases the amount of Ca^{++} bound to middle lamella-cell wall material (Buescher and Hudson, 1986), demethylation of pectins is also increased when exposure to Ca^{++} is delayed (Hudson and Buescher, 1985). Therefore, Ca^{++} appears to have at least two major functions in protecting against tissue softening; crosslinking of pectin macromolecules (Grant et al., 1973; Van Buren, 1979) and reducing demethylation of pectins.

McFeeters et al. (1985) suggested that blanching at temperatures insufficient to inactivate PME may have altered the DE of pectins by creating variable accessibility of methylated sites to PME. Rather than Ca^{++} directly inhibiting PME activity, the interaction of Ca^{++} with pectins and possibly other cell wall components might also form physical barriers that limit the access of methylated sites to PME. Since most demethylation occurs rapidly after initiating brining (Hudson and Buescher, 1985; McFeeters et al., 1985; Tang and McFeeters,

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Thermo-Kinetic Modeling of Peroxidase Inactivation during Blanching-Cooling of Corn on the Cob

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ABSTRACT

A thermo-kinetic model of peroxidase inactivation in corn on the cob during blanching-cooling is developed. Heat transfer during the blanching-cooling step is modeled as a conduction process in well stirred media of constant temperature. Corn on the cob is considered as a finite cylinder with a single thermal diffusivity value. The kinetic parameters k_r and E_a are calculated by using an unsteady state trial and error procedure. The model allows the calculation of the peroxidase activity retention for the kernel, outer cob, and central cob. Theoretical results are compared against experimental data showing a very good agreement.

INTRODUCTION

BLANCHING is an important heat process in the preparation of corn on the cob destined for freezing. Blanching is effected by heating the corn on the cob rapidly to a predetermined temperature, holding it at this temperature for a certain time, and then rapidly cooling the food. It is recognized that the difficulty in blanching corn on the cob comes from its size and the existence of three different structural areas. Long processing times are necessary to achieve complete enzyme inactivation. Often the kernels are overblanched with subsequent quality loss. Optimum processing conditions are a trade-off between preserving the organoleptic, structural and nutritional quality of the kernels and an acceptable stability during storage resulting from enzyme inactivation.

Peroxidase content has long been considered as a good blanching index due to the enzyme resistance to heat (Lee and Hammes, 1979). Recently, lipoxigenase content has been considered, for several products, as a more adequate blanching index (Adams, 1983). Thermal inactivation and residual distribution of both enzymes in corn on the cob have been presented in a recent work (Garrote et al., 1985). Several authors have reported results involving mathematical predictions of the enzyme inactivation process in green peas (Svensson and Eriksson, 1974; Gilbert et al., 1980), broccoli (Singh and Chen, 1980) and corn on the cob (Lee and Hammes, 1979). The latter authors used a simplified model of the heating step without taking into account the enzyme inactivation during the cooling step.

The present study was undertaken to determine the kinetic parameters, k and E_a, of the unsteady-state peroxidase inactivation process (Lenz and Lund, 1980) and the modeling of the enzyme activity retention during the blanching-cooling process.

THEORY

ENZYME INACTIVATION can be assumed as a first order reaction. Then,

$$\frac{dx}{dt} = -k\,x \qquad (1)$$

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The reaction rate constant, k, is temperature dependent, varying at each point of the corn on the cob, according to the Arrhenius equation. Considering that inactivation takes place during the heating and cooling steps, one obtains after integration:

$$x = \exp\left\{-k_{ref}\left\{\int_o^{th}\exp\left[-\frac{E_a}{R_g}\left(\frac{1}{T_h}-\frac{1}{T_{ref}}\right)\right]dt + \int_{th}^{th+tc}\exp\left[-\frac{E_a}{R_g}\left(\frac{1}{T_c}-\frac{1}{T_{ref}}\right)\right]dt\right\}\right\} \qquad (2)$$

In Eq. (2), k_r and E_a along with the temperature profiles during the heating and cooling steps, must be determined in order to calculate the enzyme activity retention.

According to Carslaw and Jaeger (1959) the homogeneous solution can be applied to a multilayer geometry provided K, ρ_ℓ and Cp are the same for each layer. As K essentially depends on ρ_ℓ and Cp depends on the water content, Gaffney et al. (1980) suggested that if ρ_ℓ and the water content are approximately the same for each component, the homogeneous solution can be applied without determining K and Cp. Working on kernels and cobs, Di Pentima et al. (1984) experimentally determined ρ_ℓ, water content and α and calculated Cp and K finding similar values for each component. They predicted temperature values during the cooling of the corn by using a model of several layers and a model of one layer and found no differences with the experimental values validating the use of the homogeneous model. Based on these results the whole corn is considered as a homogeneous finite cylinder with a single thermal diffusivity value. It is assumed that the cylinder heats by conduction and the resistance to energy transfer at the surface can be neglected (Biot > 100). The initial and boundary conditions of the problem are $T = T_i$ and $T = T_s$, respectively.

The solution of the transient heat transfer problem for the heating of a homogeneous finite cylinder under the above assumptions is (Carslaw and Jaeger, 1959):

$$\frac{T_h - T_i}{T_s - T_i} = 1 - \frac{8}{\pi}\sum_{n=1}^{\infty}\sum_{m=1}^{\infty}\frac{(-1)^{n+1}}{(2n-1)}\cos\left(\frac{(2n-1)\,\pi\,Z}{2\ell}\right)\cdot\exp\left[-\tau_h(2n-1)^2\left(\frac{\pi R}{2\ell}\right)^2\right]\cdot\frac{J_o(r\,\alpha_m)}{\alpha_m\,J_1(R\,\alpha_m)}\cdot\exp\left[\tau_h\,\alpha_m^2\right] \qquad (3)$$

After the heating step, the cylinder is immediately cooled by immersion in a well stirred water bath. In order to calculate the temperature profile during the cooling step, the following transient heat transfer problem must be solved:

$$\frac{\partial T}{\partial t} = \alpha\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial}{\partial r}\right)+\frac{\partial^2 T}{\partial Z^2}\right];\quad \begin{matrix}0 \le r < R\\ -\ell < Z < \ell\end{matrix};\ t > 0 \qquad (4)$$

Initial condition:

$$T = T_s - (T_s - T_i)\,\Phi \tag{5}$$

and

$$\Phi = \left\{\frac{4}{\pi}\sum_{n=1}^{\infty}\frac{(-1)^{n+1}}{(2n-1)}\cos\left[\frac{(2n-1)\,\pi\,Z}{2\ell}\right]\cdot\exp\left[-\tau_h(2n-1)^2\left(\frac{\pi R}{2\ell}\right)^2\right]\right\}\cdot\left\{\frac{2}{R}\sum_{m=1}^{\infty}\frac{J_o(r\,\gamma_m)}{\gamma_m\,J_1(R\,\gamma_m)}\exp\left(-\tau_h\,\gamma_m^2\right)\right\} \tag{6}$$

Boundary conditions:

$$\left.\begin{aligned}\frac{\partial T}{\partial r} &= 0 \text{ at } r = 0\\ \frac{\partial T}{\partial Z} &= 0 \text{ at } Z = 0\\ T &= T_w \text{ at } r = R\\ T &= T_w \text{ at } Z = \ell\end{aligned}\right\} \tag{7}$$

The solution is:

$$\frac{T_c(r,Z,t) - T_w}{T_s - T_i} = 8\sum_{m=1}^{\infty}\sum_{j=1}^{\infty}\exp\left[-\alpha\left(\frac{\beta_j^2}{R^2} + \frac{(2m-1)^2\,\pi^2}{4\,\ell^2}\right)t\right]\cdot\frac{J_o(r\,\beta_j)}{(2m-1)\,\pi\,\beta_j\,J_1(R\,\beta_j)}\cdot\sin\left[\frac{(2m-1)\,\pi\,(Z+\ell)}{2\ell}\right]\cdot\left[\frac{T_s - T_w}{T_s - T_i} - \exp\left[-\tau_h\,(\beta_j^2 + (2m-1)^2\,\frac{\pi^2 R^2}{4\ell^2}\right]\right] \tag{8}$$

This solution agrees with the one presented by Lenz and Lund (1977). The above solution for the cooling of the cylinder contains the same assumptions as the one for the heating problem. Equations (3) and (8) describe the heating and cooling steps, the thermal diffusivity being the adjusting parameter.

In order to calculate the kinetic parameters, k_r and E_a in Eq. (2), an iterative method was used (Lenz and Lund, 1980); this method requires the calculation of the volumetric average enzyme activity retention. From Eq. (2):

$$\bar{x} = \frac{\int_v x\,dV}{\int_v dV} \tag{9}$$

For a finite cylinder, and assuming the volume of the corn on the cob as a constant during the heating and cooling steps:

$$\bar{x} = \int_{\zeta=-1}^{1}\int_{\rho=0}^{1} x(\rho,\zeta)\,\rho\,d\rho\,d\zeta \tag{10}$$

Performing a numerical integration (Kreyszig, 1979) one obtains:

$$\bar{x} = \sum_{j=1}^{n} A_j \sum_{i=1}^{k} B_i\,x\,(\rho_i,\zeta_j) \tag{11}$$

This expression has the same form as the one obtained by Lenz and Lund (1977). The values of coordinates and coefficients in Eq. (11) were obtained from Abramowitz and Stegun (1964). For the present application, it was found that 18 points (n = 3, k = 6) were adequate to obtain the value of the integral within 0.1% of that when more points were used. The final result is:

$$\bar{x} = 2\sum_{j=1}^{3} A_j \sum_{i=1}^{6} B_i\,x\,(\rho_i,\zeta_j) \tag{12}$$

This equation allows the application of the transient trial and error method proposed by Lenz and Lund (1980) in order to estimate the kinetic parameters k_r and E_a.

The next step is to calculate the enzyme activity retention for each zone of the corn. The kinetic model is to be applied to a multilayer finite cylinder. The average zone enzyme activity retention is calculated by taking the volume average of the point enzyme retention given by Eq. (2) for each zone of the corn.

Equation (9) is now:

$$\bar{x}_{zone} = \frac{\int_{\zeta=-1}^{1}\int_{r=a}^{b} x(r,\zeta)\,r\,dr\,d\zeta}{\int_{r=a}^{b} r\,dr} \tag{13}$$

On defining:

$$g(r,\zeta) = x(r,\zeta)\cdot r \tag{14}$$

Eq. (13) becomes:

$$\bar{x}_{zone} = \frac{\int_{\zeta=-1}^{1}\int_{r}^{b} g(r,\zeta)\,dr\,d\zeta}{\int_{r=a}^{b} r\,dr} \tag{15}$$

In order to normalize, the radial variable can be changed to: for r = a , $\rho^* = 0$; for r = b , $\rho^* = 1$. Now

$$\rho^* \triangleq \frac{r - a}{b - a} \tag{16}$$

Equation (15) becomes:

$$\bar{x}_{zone} = \frac{2}{(b+a)}\int_{\zeta=-1}^{1}\int_{\rho^*=0}^{1} g(\rho^*,\zeta)\,d\rho^*\,d\zeta \tag{17}$$

The integral can be evaluated by using the Gauss numerical integration (Kreyszig, 1979). Eq. (17) becomes:

$$\bar{x}_{zone} = \frac{2}{(b+a)}\sum_{j=1}^{n} A_j \sum_{k=1}^{m} C_k\,g(\rho_k^*,\zeta_j) \tag{18}$$

Coefficients A_j and coordinate values are the same as those used in Eq. (11). Applying the same considerations as for Eq. (12) the final result is:

$$\bar{x}_{zone} = \frac{6}{(b+a)}\sum_{j=1}^{3} A_j \sum_{k=1}^{6} C_k\,g(\rho_k,\zeta_j) \tag{19}$$

C_k values and radial coordinates ρ_k are from Abramowitz and Stegun (1964). The starting point in evaluating $g(\rho_k^*,\zeta_j)$ is from Eq. (16):

$$r_k^{zone} = a + (b - a)\,\rho_k^* \tag{20}$$

By replacing the values of r_k^{zone} and ζ_j in Eq. (3) and (8) one

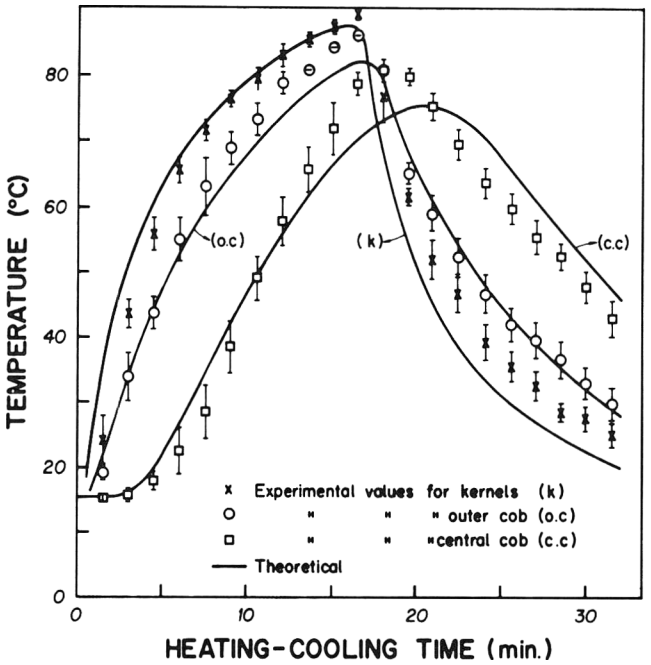

Fig. 1—Experimental and theoretical temperature profiles for kernels, outer cob and central cob during thermal processing (Heating: 100°C, Cooling: 2°C). Bars represent average standard deviations. Thermocouples were located at the center and at 1 cm and 1.7 cm from the center.

obtains the heating and cooling temperature profiles. By replacing these expressions in Eq. (2) multiplied by r_k^{zone} given by Eq. (20) one obtains the values of $g(r_k^{zone},\zeta_j)$. In this way one can evaluate the zonal average enzyme activity retention given by Eq. (19).

MATERIALS & METHODS

SWEET YELLOW corn on the cob, Jubilee variety, supplied from growers of the San Javier area (Santa Fe Province, Argentina) was used in this study. The water content over six determinations was in the range 71.45 + 2.3%. Ears were peeled, selected, washed, and cut into pieces 12.5 cm in length. The mean diameter of the samples was 4.6 ± 0.18 cm.

Enzyme inactivation

Sets of ten selected pieces were blanched in a pilot scale steam blancher at 80°, 90°, and 100°C for 4, 8, 12, and 16 min. The pieces were cooled by immersion in a well-stirred water bath held at 2°C. Cooling times were the same as blanching times. Temperature measurements were carried out by means of a set of three thermocouples held at the center of the piece and at 1 cm and 1.7 cm from the center. Experimental procedures used were described by Garrote et al. (1985).

Enzyme activity determinations

Enzyme activity was determined on fresh and blanched corn in triplicate. Determinations were made in the kernels (removed from the ear) ($d \geq 2.8$ cm), in the external part of the central cob (1.3 cm $\leq d \leq 2.8$ cm), in the outer part of the central cob ($d \leq 1.3$ cm) and in a pooled sample collected from all sections of the corn on the cob. A spectrophotometric method was used to determine enzyme activity (Vetter et al., 1958). Experimental procedures used were described by Garrote et al. (1985).

RESULTS & DISCUSSION

Heating-cooling temperature profiles and thermal diffusivity values

The experimentally determined temperature values for the three areas of the corn on the cob are shown in Fig. 1, along with the theoretical curves [Eq. (3) and (8)] which best fitted the data. The associated values of thermal diffusivities are $\alpha_{heating} = 1.4 \times 10^{-3}$ cm²/sec and $\alpha_{cooling} = 1.1 \times 10^{-3}$ cm²/sec. Standard deviations between experimental and theoretical temperature values were for the heating step (steam at 100°C): $\sigma_{kernel} = 3.15$°C, $\sigma_{outer\ cob} = 2.7$°C and $\sigma_{central\ cob} = 3.77$°C and for the cooling step (water at 2°C): $\sigma_{kernel} = 2.58$ °C, $\sigma_{outer\ cob} = 1.3$°C and $\sigma_{central\ cob} = 5.77$°C.

Table 1—Percent deviation between theoretical and experimental temperature profiles (Fig. 1) for the different zones of the corn on the cob.

Step	% Deviation		
	Kernel	Outer cob	Inner cob
Heating (100°C–16 min)	13.63	7.64	7.01
Cooling (2°C–16 min)	12.31	4.32	9.77

Table 2—Experimental average peroxidase retention in corn on the cob for several processing conditions (T_i = 15°C, T_w = 2°C).

Processing time (min)	Experimental average retention: $\bar{x}_{exp} \pm \sigma_n$ (%)		
	T_s = 80°C	T_s = 90°C	T_s = 100°C
8	79.00 ± 7.90	64.49 ± 3.85	49.51 ± 3.49
16	58.60 ± 5.86	42.44 ± 2.12	38.45 ± 3.84
24	47.81 ± 4.64	30.90 ± 3.09	20.32 ± 1.62
32	43.87 ± 6.58	26.89 ± 2.15	11.73 ± 1.4

Experimental data in Fig. 1 are for heating and cooling steps of 16 min each. Similar results were obtained for the heating medium at 80°C and 90°C.

Thermal diffusivity was also estimated from Riedel's equation (Gaffney et al., 1980). For the heating step, considering a mean temperature of 57.5°C (T_i = 15°C ; T_s = 100°C) and a water content of 72%, the calculated thermal diffusivity value was $\alpha^h_{calc} = 1.4 \times 10^{-3}$ cm²/sec which agreed with the estimated value from time-temperature data. However, for the cooling step with a mean temperature of 51°C (T_c = 2°C, T_h = 100°C) and a water content of 72%, the calculated thermal diffusivity value was $\alpha^c_{calc} = 1.39 \times 10^{-3}$ cm²/sec which is higher than the estimated value. It is possible that the difference can be accounted for by the change in volume in the corn on the cob during the cooling step. This modifies the diameter of the material which in turn will influence the estimated value. Di Pentima et al. (1983), working in a temperature range 0–30°C, found values of 1.28×10^{-3} cm²/sec for kernels and 1.24×10^{-3} cm²/sec for cobs.

In this study values of 1.4×10^{-3} cm²/sec and 1.1×10^{-3} cm²/sec were used for predicting the temperature profiles during the heating and cooling steps. Model equations were also compared to the data by means of percent deviation (Heldman, 1974).

Table 1 shows the pertinent results. The values are very reasonable considering the biologic and structural variability of the material, the nonuniform geometry and the experimental problems in placing the temperature probes in the right places and in measuring the temperature values.

Estimation of the kinetic parameters k_r and E_a

As seen in Eq. (2), in addition to the temperature profiles, the kinetic parameters k_r and E_a are determined applying the transient trial and error method proposed by Lenz and Lund (1980). Table 2 shows the experimental average peroxidase retention values. Table 3 shows the results of the iterative procedure and Fig. 2 shows the iterative procedure for t = 32 min.

The calculated values of E_a are similar to those obtained by Yamamoto et al. (1962) for corn kernels, by Garrote et al. (1985) for corn on the cob and by Ling and Lund (1978) for horseradish peroxidase.

The values of k_r listed in the Table 3 are similar to those determined by Garrote et al. (1985) using a stationary method, for processing times of 16 and 24 min. For processing times

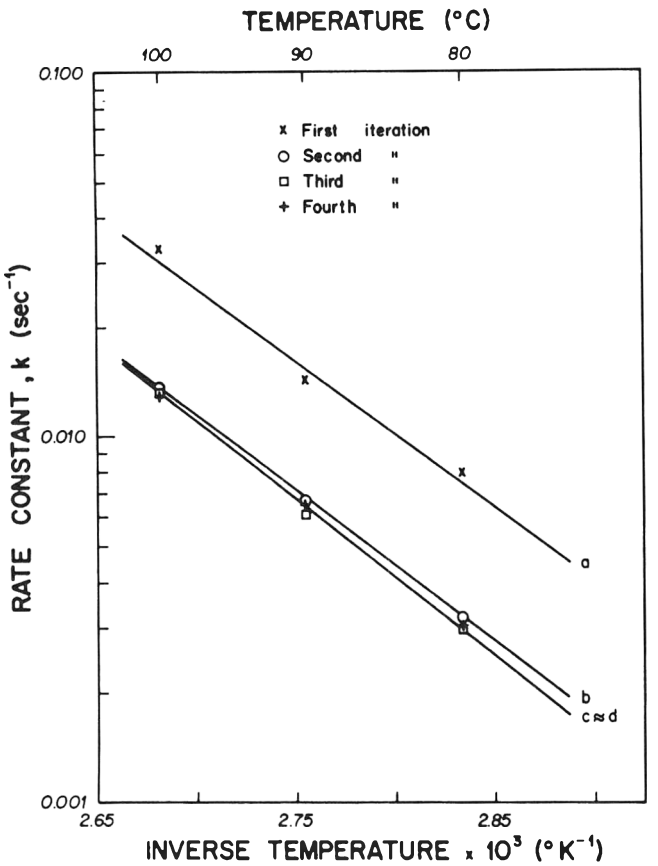

Fig. 2—Arrhenius plot for peroxidase inactivation in whole corn on the cob, illustrating iterations in trial and error procedure to obtain a kinetic model using the unsteady state method (Reaction time = 32 min).

Table 3—k, E_a and $\bar{x}$ results by applying the trial and error method

Heating medium temperature T_S(°C)	Processing time t(min)	E_a (kcal/mol)	$\bar{x}$ (%)	K_{final} (sec^{-1})
	32	19.8	44.19	0.0031
80	24	19.8	47.82	0.0050
	16	18.2	58.17	0.0065
	32	19.8	27.36	0.0064
90	24	19.8	30.74	0.0113
	16	18.2	42.19	0.0155
	32	19.8	11.70	0.0130
100	24	19.8	20.05	0.0213
	16	18.2	37.34	0.0231

of 32 min a considerable deviation can be observed. This is probably due to the presence of a heat resistant isoenzyme, specially at 90°C and 100°C, as reported by Garrote et al. (1985). As a consequence, the mean values of k_r and E_a for processing times of 16 and 24 min are assumed valid for the kinetic model. Then: $k_{ref_{80°C}} = 0.0057\ sec^{-1}$, $k_{ref_{90°C}} = 0.0134\ sec^{-1}$, $k_{ref_{100°C}} = 0.0222\ sec^{-1}$ and $E_a = 19$ Kcal/mol. By replacing these values in Eq. (12) one obtains the curves representing the average enzyme activity retention for different processing conditions. Figure 3 shows the theoretical and experimental values. Good agreement can be observed for processing times up to 24 min.

Prediction of peroxidase residual activity in kernel, outer cob and central cob sections during blanching-cooling

Figure 4 presents the theoretical and experimental values of peroxidase retention for different processing conditions and for each zone of the corn on the cob. A good agreement can be

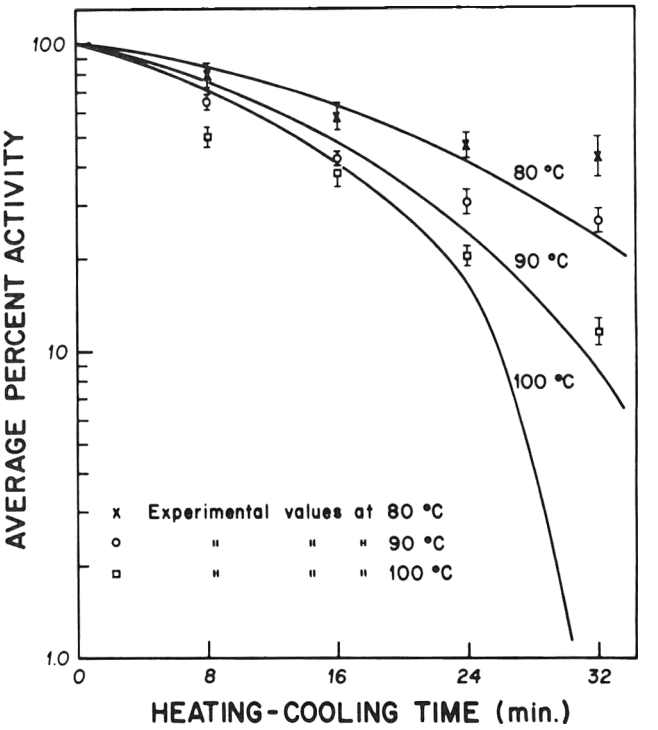

Fig. 3—Thermal inactivation curves for corn on the cob peroxidase at several processing temperatures. Solid lines represent the theoretical values.

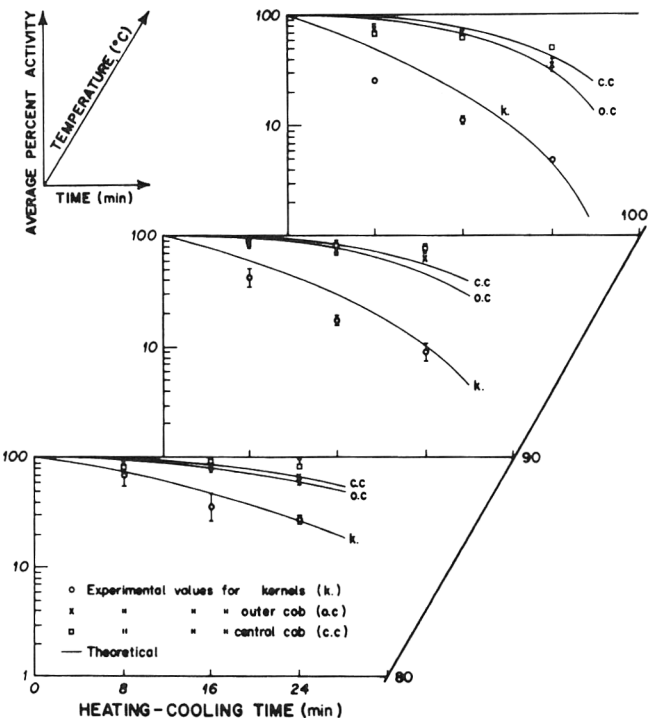

Fig. 4—Thermal inactivation curves for corn on the cob peroxidase in kernels, central cob and outer cob for several processing temperatures.

observed in outer and central cob for the three processing temperatures as well as for kernels at 80°C. The results show that the proposed thermo-kinetic model works very well considering the biologic variability of the material used as well as the nonuniform geometry of the real corn on the cob. The goodness of the model can also be visualized by comparing experimental values of enzyme activity retention with the

Table 4—Percent relative error between the experimental global average enzyme activity retention and the calculated value by using Eq. (21).

Temperature	80°C			90°C			100°C		
Time (min)	8	16	24	8	16	24	8	16	24
Percent relative error	1.98	3.55	2.63	6.48	7.57	5.15	6.51	11.23	1.49

corresponding calculated weighted average values for each zone according to:

$$\text{Experimental weighted retention} = 0.6\,\bar{x}_{\text{kernel}} + 0.27\,\bar{x}_{\text{outer cob}} + 0.13\,\bar{x}_{\text{inner cob}} \quad (21)$$

where 0.6, 0.27, and 0.13 are the weight factors for kernels, outer cob, and inner cob. Table 4 shows the percent relative error considering the global experimental retention and the one calculated by using Eq. (21).

CONCLUSIONS

A THERMO-KINETIC MODEL describing the peroxidase inactivation in corn on the cob during the blanching-cooling process has been developed. Estimated thermal diffusivity values for the heating and cooling steps were 1.40×10^{-3} cm^2/sec and 1.10×10^{-3} cm^2/sec, respectively. The determined $k_{100°C}$ and E_a values for the peroxidase enzyme were 0.022 sec^{-1} and 19 Kcal/mol, respectively. The model allows us to make very good estimates of the peroxidase activity retention in kernels, outer cob and inner cob at 80°C, 90°C and 100°C for different processing times.

NOMENCLATURE

a	radial position
b	radial position
B_i	weighting factor
Biot	Biot number
C	enzyme concentration at time "t"
C_o	initial enzyme concentration
C_k	weighting factor
C_p	specific heat
d	diameter
E_a	activation energy
J_o	zero order Bessel function
J_1	first order Bessel function
K	thermal conductivity
k	rate constant at temperature T
k_{ref}	rate constant at temperature T_{ref}
ℓ	cylinder length
r	radial coordinate
R	cylinder radius
R_g	gas law constant
t	time
t_c	cooling time
t_h	heating time
T	temperature
T_c	cooling temperature
T_h	heating temperature
T_i	initial temperature
T_{ref}	reference temperature
T_s	heating medium temperature
T_w	cooling medium temperature
V	volume
x	relative retention $= \left(\frac{C}{C_o}\right)$
$\bar{x}_{exp}$	mean experimental enzyme activity retention
$\bar{x}_{zone}$	mean zonal enzyme activity retention (%)
Z	axial coordinate
α	thermal diffusivity
α^c_{calc}	calculated thermal diffusivity for the cooling step
α^h_{calc}	calculated thermal diffusivity for the heating step
β_j	roots of $J_o(\beta) = 0$
γ_m	roots of $J_o(\gamma) = 0$
ζ	dimensionless axial length
ρ_ℓ	density
ρ	dimensionless radius
ρ^*	normalized radial variable
σ	standard deviation
τ_h	heating Fourier number

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Effects of Simulated Retail Display and Overnight Storage Treatments on Quality Maintenance in Fresh Broccoli

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ABSTRACT

The quality of fresh broccoli during simulated retail display was evaluated using contact (ice) and air cooling separately and in combination. Chlorophyll and ascorbic acid were best maintained with air cooling, while weight was best maintained with ice cooling alone. In air-cooled display units, ice placed both on top of and beneath broccoli heads resulted in more rapid loss of chlorophyll and ascorbic acid than did bottom-icing alone. Bottom-iced broccoli in air-cooled units kept best overnight if left in place and covered with wet burlap. Removal to a 4°C walk-in cooler each night resulted in good color retention and marketability but weight loss after 4 days was significantly higher.

INTRODUCTION

THE STORAGE LIFE of produce at the retail level is partially determined by handling practices from the time of harvest until it reaches retail outlets. For example, broccoli (*Brassica oleracea* L. Italica group) keeps best if it is hydrocooled soon after harvest (Gerwe and Slade, 1952). If it is stored at 0°C and 95% relative humidity (R.H.), its green color is maintained much longer than when unrefrigerated (Wang and Hruschka, 1977). Also, numerous studies have shown that broccoli benefits substantially from controlled or modified atmosphere storage (Wang, 1979). However, broccoli grown in areas close to urban centers is often transported directly to the retail outlet from the areas of harvest, without the benefits of good postharvest handling procedures. Under these circumstances, the method of handling at the retail level is especially critical in reducing losses both before and after consumer purchase.

In investigating the effects of various treatments on the color of fresh broccoli, Shewfelt et al. (1983) demonstrated that after 4 wk, broccoli stored at 0–1°C and capped with flake ice, began to show better chlorophyll retention than noniced broccoli stored at the same temperature. While these results help to demonstrate the long-term storage potential for broccoli, they do not help in understanding the problem of short-term management under retail conditions. For example, the chlorophyll data are based on an analysis (Batal et al., 1982) which includes 5–6 cm of stem tissue which would not be exposed to the same harsh conditions as the florets and which would not be readily seen by the retail shopper. Also, in terms of present retail practice, it is unlikely that broccoli would be stored at the temperatures or for the time periods tested.

Retail display practices for broccoli vary greatly within the industry. In a survey of 13 British Columbia supermarkets, only six utilized air-cooled (mechanically refrigerated) display cases for broccoli, and in one of these, the display surface was elevated above the refrigerated zone. Seven stores utilized ice for broccoli and only two of these also used air-cooled refrigerated cases for this product. Ice was placed as a bed under the broccoli or distributed both above and below the heads. The size of ice particles varied from a fine crush to large cubes. Three markets routinely sprayed broccoli with water while it was being displayed, but only two, with air-cooled cases, left their broccoli in the display case overnight. Both stores covered the broccoli with wet burlap for this period.

Lewis (1957), who has provided good general guidelines for maintaining product quality in retail stores, stressed the importance of careful handling and recommended that produce which is left in the display case overnight be covered with wet canvas or heavy paper to lessen wilting. He showed that broccoli sprinkled with tap water three times per day will increase in weight by 5% after 3 days, while broccoli without sprinkling will decrease in weight by 1% over the same period.

The present study was undertaken to provide a physiological basis for practical recommendations concerning retail handling of broccoli. Ascorbic acid is a well-known and nutritionally important component of broccoli which, by virtue of its instability, serves as an excellent indicator of produce quality (Ezell and Wilcox, 1959). Determining chlorophyll content provides a sensitive measure of greenness in broccoli (Lebermann et al., 1968) and monitoring weight change gives an indication of loss of moisture and turgor and is a direct measure of total loss in marketable produce. Thus, by subjecting broccoli to a variety of cooling, icing and handling treatments simulating those observed in retail practice and by monitoring ascorbic acid, chlorophyll and weight, an objective measure of treatment effects will be obtained. Comparing the results with simple sensory evaluations for color and odor (Wang, 1979) will provide an indication of treatment differences observable by produce managers.

MATERIALS & METHODS

DISPLAY PRACTICES and overnight storage for broccoli were evaluated in two separate experiments. In each experiment, the broccoli was transported immediately after harvest to the laboratory where it was cut approximately 21 cm in length, weighed and randomly assigned to treatments and replicates. The broccoli was held for 20 hr at 0.5 ± 0.5°C and 95–97% R.H. prior to placement in a mechanically refrigerated, forced-air display case (open top, Model STVTO-12 Hussman Refrigeration Co. Ltd., Brantford, Canada) at 7–9°C (unloaded) or a specially constructed nonrefrigerated, insulated display unit of similar shape and size (first experiment only).

In the first experiment (August, 1983), the broccoli in both display units was subjected to three different icing treatments: ice placed both on top of and beneath the samples, ice placed only beneath the samples, and no ice applied. In the treatments using ice, the butts of the broccoli were embedded in an 8–10 cm layer of cubed ice (1.5 × 1.5 × 1.0 cm) which was replenished as necessary during the day but was allowed to diminish unchecked overnight. At this time, burlap covering was not used. The broccoli was left in the units for the duration of the experiment. Each treatment was replicated three times. Six heads per treatment were utilized on the nonrefrigerated (no air cooling) unit and eight heads per treatment, to permit sampling after 4 days, were used in the forced-air (refrigerated) display case. Based on the findings of a preliminary test, the first experiment was terminated in two stages: after 4 days for the treatments without air cooling, with an air-cooled control, and after 7 days for the remainder of the air-cooled treatments. Each broccoli head was weighed initially and again on removal from the display units to determine the effect of different treatments on weight loss. The broccoli in the forced-air unit was also weighed after 4 days. Thermistor probes were inserted into the broccoli butts of one sample in each treatment to record temperature fluctuations. The broccoli was evaluated daily for color and

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odor, using a rating scale developed by Wang (1979). Color values of 10, 8, 6, 4, 2, and 0 were assigned for dark green, light green, slightly yellow, moderately yellow, severely yellow, and complete yellow respectively. The same values were used for odor rating, where 10 was normal and successively lower ratings represented trace off-odor, slight off-odor, moderate off-odor, severe off-odor, and nauseating odor respectively. The ratings were performed by the coauthors and an assistant under cool white fluorescent lighting and were used to supplement the chemical and weight data and to provide an indication of treatment differences observable by produce managers.

In the second experiment (July, 1984), the most successful display method of Experiment One, refrigerated, forced-air display with bottom icing, was employed. Again, samples consisting of six heads were replicated three times for each of the three treatments. The overnight storage treatments were as follows: unprotected in display cases; covered with wet burlap in display cases; or removed to a 4 ± 1°C coldroom in ventilated, waxed cardboard cartons. This storage room temperature was selected since it approximated the mean supermarket storage temperature observed during the earlier survey. The color, odor and temperature of the broccoli were monitored as in the first experiment. However, chlorophyll was not measured directly, but color quality was established on the basis of visual ratings only, since there was a good correlation between color rating and chlorophyll in the first experiment ($r = +0.89$). All other procedures were the same for both experiments.

Chemical analyses were performed on the day of harvest and upon termination of each experiment to evaluate the loss of green coloration (chlorophyll, Experiment One only) and Vitamin C (ascorbic acid). The harvest day analyses were performed on six heads of similar maturity and size as those used in the experiment. Samples were obtained by the removal and thorough mixing of the buds of heads in each treatment. In the first experiment, four heads per replicate from the forced-air display case were combined and analysed on the fourth day and the remaining four heads were analyzed on the seventh day.

Total chlorophyll was analyzed according to the AOAC method (AOAC, 1980). Approximately 2.5g of tissue were ground using $CaCO_3$ and extracted with 85% acetone (final volume of 50 mL). The absorbance of the extract was measured at 660 nm using a Pye Unicam SP650 visible spectrophotometer.

Ascorbic acid was determined using a Beckman gradient Liquid Chromatograph Model 322. The sample was prepared by grinding approximately 1.0g of tissue in 10 mL of 0.4% oxalic acid. The homogenate was centrifuged and the aqueous portion extracted with chloroform, then with diethyl ether, filtered, and a 20 μL sample injected on a 250 mm × 4.6 mm Altex Ultrasphere-ODS column. A mobile phase of 2% aqueous $NH_4H_2PO_4$ with U.V. detection of 245 nm was used (Perrin, 1983).

Analysis of variance and Duncan's Multiple Range test were used to statistically analyse the data (Duncan, 1975).

RESULTS

THE INITIAL ASCORBIC ACID content of the broccoli buds was 143 mg/100g (s.d. = 6.58) and 185 mg/100g (s.d. = 1.33) for Experiments One and Two respectively and initial chlorophyll was 74 mg/100g (s.d. = 19.6) for Experiment One. The variability in chlorophyll did not noticeably affect the marketable appearance.

Experiment One

After 4 days, the treatments without air cooling were terminated. At this time, these samples had become slightly flaccid and heads had lost compactness with some florets opening or showing noticeable yellowing. Analysis showed that significantly ($P<0.05$) more ascorbic acid and chlorophyll were retained in samples placed on the forced-air unit compared to those on the unit without forced-air movement (Table 1). The percentage of ascorbic acid lost from the samples without air cooling was more than twice that lost from the air-cooled samples. The loss of chlorophyll was about 1.7 times greater in the unit without forced-air. After 7 days the remaining air-cooled samples were exhibiting yellowing and a loss of turgor similar to the treatments terminated earlier.

Loss of ascorbic acid, chlorophyll and weight from the samples treated without ice was significantly ($P<0.05$) greater than in those treatments which utilized ice (Table 1). The loss of ascorbic acid was greatest with samples placed on the unit without forced-air cooling and treated either with bottom ice or with no ice. Significantly ($P<0.05$) more ascorbic acid was retained by top and bottom-iced samples without air cooling. The least loss occurred in samples placed on the forced-air unit and iced on the bottom only. After 7 days, the ascorbic acid in air-cooled samples with bottom ice was still significantly ($P<0.05$) greater than that in samples iced both top and bottom.

Chlorophyll was best retained in samples which were placed on the forced-air unit and bottom iced. This is consistent with the results found for ascorbic acid. The greatest ($P<0.05$) chlorophyll loss (8.2 mg/100g remained) was observed after 4 days in treatments without air-cooling and without ice (Table 1). The observations are supported by the sensory ratings for color (Table 2). Color ratings and chlorophyll measurements were closely correlated ($r = +0.89$) as mentioned above. The most acceptable broccoli odor was reported for samples in the forced-air unit and bottom iced (Table 2). In air-cooled samples, ratings for odor and color were closely correlated.

The least weight loss was observed in samples which were not air-cooled. After 4 days, top and bottom icing was better than bottom icing which was better than no ice for weight retention. However, after 7 days, the air-cooled treatments showed no significant ($P<0.05$) difference in weight loss between bottom iced and top and bottom iced samples.

Average core temperatures and ice usage for the various treatments are shown in Table 3. In the forced-air display case, ice melted much faster than in the case without forced-air. Ice on the treatment which received ice both top and bottom, was usually completely melted by 08:00 hr and the temperature was higher than that of the bottom iced treatment which always had a small amount of ice remaining from the previous day. On the unit with refrigerated, forced air, the data show that air cooling accounts for almost half the total cooling achieved with both air and ice cooling.

Experiment Two

Analysis showed a significant difference in ascorbic acid loss after 4 days between broccoli that was removed in waxed cartons to a 4°C cooler and broccoli left uncovered in the display cases overnight (Table 4). Chlorophyll was not analyzed; however, visual ratings showed no significant ($P<0.05$) treatment color differences after 4 days.

Treatments were distinguished most clearly on the basis of weight loss. Broccoli which remained in the display case overnight covered with wet burlap retained significantly more weight than broccoli which was either removed to the cooler or left uncovered in the display case (Table 4). Uncovered broccoli utilized about 13.3 kg of ice/treatment, a slight decrease from that used for the same treatment in Experiment One.

DISCUSSION

THIS STUDY provides physiological evidence which supports some of the earlier recommendations of Lewis (1957), particularly as they apply to refrigerated display cases. Higher levels of ascorbic acid and chlorophyll which were observed on the forced-air display unit in Experiment One were probably a result of the lower core temperatures which were observed. Even at the warmest part of the day, when room temperatures averaged about 25°C, the treatment which received no ice on the forced-air unit attained a maximum core temperature of only 11.0°C, while the treatment without ice on the unit without forced-air reached 19.7°C. On the unit without forced-air, top and bottom icing produced a greater temperature reduction than bottom icing alone, as might be expected. However, on the forced-air unit, the air circulation apparently resulted in a high rate of heat transfer to the top ice, which consequently melted very rapidly. Overnight, it appeared that water from the top ice dripping on the bottom ice caused the bottom ice

Table 1—Effect of air cooling, icing and cooling × icing treatments on ascorbic acid and chlorophyll content and weight loss in broccoli during retail display (Experiment One)[a]

		Means					
		Ascorbic acid (mg/100g)		Chlorophyll (mg/100g)		Loss of weight (g)	
Factor	Treatment	Day 4	Day 7	Day 4	Day 7	Day 4	Day 7
Cooling	Air-cooled	84.6a(205.4)	53.0	42.4a(3.6)	35.4	66.1b(79.6)	90.1
	None	36.2b	–	22.9b	–	11.5a	–
Icing	Top & bottom	66.9a(115.8)	–	36.2a(7.8)	–	16.7a(71.8)	–
	Bottom	71.3a	–	37.6a	–	38.2b	–
	No ice	43.1b	–	24.2b	–	61.6c	–
Cooling	Air × T&B	70.3b(107.8)	35.8b(39.9)	35.0b(9.0)	27.3b(5.6)	33.1b(165.4)	79.9a(321.9)
	× Bottom	108.2a	69.4a	52.1a	41.9a	73.5c	78.7a
	× None	75.4b	53.9ab	40.2b	36.9a	91.5c	105.4a
	None × T&B	63.6b	–	37.5b	–	0.3a	–
	× Bottom	34.3c	–	23.2c	–	2.8a	–
	× None	10.6c	–	8.2d	–	31.6b	–

[a] Means in columns within factors followed by the same letters were not significantly different; separation of means by Duncan's Multiple Range Test ($p<0.05$); initial mean concentrations of ascorbic acid and chlorophyll were 143 mg/100g and 74 mg/100g respectively; initial mean head weight was 212.1g; Error Mean Squares in parentheses.

Table 2—Effect of cooling, icing and cooling × icing treatments on mean ratings for color and odor in broccoli during retail display (Experiment One)[a]

		Mean ratings			
		Color		Odor	
Factor	Treatment	Day 4	Day 7	Day 4	Day 7
Cooling	Air-cooled	9.1a(0.007)	5.2	8.0a(0.26)	7.6
	None	4.8b	–	6.7b	–
Icing	Top & bottom iced	8.5a(0.44)	–	8.0(0)	–
	Bottom iced	7.3b	–	8.0	–
	No ice	5.0c	–	6.0	–
Cooling	Air × Top & bottom	9.2ab(0.44)	4.7b(0.32)	8.0(0)	6.7b(1.19)
× Icing	× Bottom	9.9a	6.8a	10.0	9.5a
	× No ice	8.1b	4.2b	6.0	6.7b
	None × Top & bottom	7.8b	–	8.0	–
	× Bottom	4.7c	–	6.0	–
	× No ice	2.0d	–	6.0	–

[a] Means in columns within factors followed by the same letters were not significantly different; separation of means by Duncan's Multiple Range Test ($P<0.05$); rating of 10 is best, zero is worst (avg. of < 6 is unmarketable); Error Mean Squares are in parentheses.

Table 3—Mean[a] *core temperatures and ice usage for cooling of broccoli in display cases (Experiment One)*

Treatments		Temperature means	Effective temp reduction	Ice usage
Air cooling	Contact cooling	°C	°C	kg/day
Yes	Top & bottom iced	3.8 ± 1.5	15.2	17.8
	Bottom iced	3.0 ± 0.2	16.0	13.6
	No ice	11.6 ± 0.6	7.4	0
No	Top & bottom iced	3.8 ± 1.8	15.2	9.5
	Bottom iced	9.2 ± 1.2	9.8	7.0
	No ice	19.0 ± 0.7	0	0

[a] Means for forced-air cooling and No air cooling are for 7 and 4 days respectively; temperatures were monitored at 08:00 hr and 16:00 hr.

Talbe 4—Effect of overnight storage treatments on ascorbic acid, color and weight loss in broccoli after four days in simulated retail display (Experiment Two)

	Means		
Treatment	Ascorbic acid (mg/100g)	Color rating	Loss of wt (g)
Uncovered in display case	140.9b	9.3a	42.6b
Covered in display case	156.5ab	9.6a	16.3a
Waxed cartons in cooler	157.3a	9.7a	33.6b

[a] Treatment means in the same column that are followed by the same letters are not significantly different (Duncan's Multiple Range Test, $p<0.05$). In color ratings, 10 was best and 0 was worst. The Error Mean Squares for ascorbic acid, color and weight loss were 49.8, 0.344, and 16.9, respectively. Initial mean head weight was 345.2g.

to melt more rapidly. Consequently, in the morning, the top and bottom iced treatment was slightly warmer than the treatment receiving only bottom ice.

Weight loss did not show the expected inverse relationship to ascorbic acid and chlorophyll in the first experiment. Normally, keeping quality would be reflected in retention of chlorophyll, ascorbic acid and moisture. Therefore, while placing cube ice on top of broccoli heads resulted in weight retention, it also caused damage to the delicate bud tissue. This tissue soon died, with resultant yellowing and loss of chlorophyll and ascorbic acid. Bottom icing, while less effective than top icing for weight retention, aided chlorophyll and ascorbic acid retention without bud damage.

The addition of water to the bud surface (rather than ice) in combination with bottom icing may be an effective means of achieving moisture retention without bud injury. However, in designing these experiments, preliminary tests, during which decay was observed and rated, confirmed Lewis' (1957) findings that excessive free moisture on the bud surface of some broccoli heads resulted in decay development, which is contributed to by the warmer temperatures. In forced-air cases with bottom icing, temperatures between 5° and 10°C were measured near the bud surface while room temperatures were near 20°C. It was noted in our supermarket survey that some produce departments routinely sprayed the broccoli with water. While some water can be tolerated and may be beneficial, spraying must be carefully regulated in view of the potential for decay development. Excessive moisture will also result in a higher rate of ice usage which may be a factor in determining the cost benefit of ice use.

The loss of chlorophyll and ascorbic acid associated with the treatments using no ice was partially a natural consequence of senescence (Shewfelt et al, 1983). However, this process was undoubtedly accelerated by moisture loss, as weight loss (loss of moisture) averaged almost 30% in these treatments. The desiccating effect of air movement in the forced-air units was responsible for generally high weight losses which reached almost 50% after 7 days in those treatments without ice.

Experiment Two demonstrated that significant differences

in weight loss may arise without noticeably affecting color rating. Thus, produce managers may be unaware of losses in produce quality. Broccoli stored overnight in the 4°C cooler lost about 5% more weight and about 4% more ascorbic acid than broccoli covered with burlap. Some researchers have suggested that temperatures between 0°C and 5°C have little effect on ascorbic acid retention (Eheart and Odland, 1972). Nevertheless, from a marketing viewpoint, treatments that help to control weight loss should probably be given primary consideration.

CONCLUSIONS

THE RESULTS indicated that the keeping quality of broccoli at the retail level was significantly enhanced by display in mechanically refrigerated cases. The benefits obtained included in the retardation of yellowing, retention of chlorophyll (green coloration) and the retention of nutritional value as measured by ascorbic acid. The highest retention of ascorbic acid and chlorophyll was observed when a bed of cube ice was placed beneath the heads (bottom iced) in combination with mechanical refrigeration. However, top and bottom icing resulted in the highest moisture retention during the first 4 days. For overnight storage, weight loss can be minimized by keeping the broccoli in mechanically refrigerated display cases on a bed of cube ice and covering with wet burlap.

This study indicates that there may be potentially harmful effects from top icing broccoli. It is possible that ice which is finely crushed or shaved and not more than a few degrees below 0°C will not damage broccoli buds. However, if the broccoli is moved from the store after only 1 or 2 days, any damage caused by large-sized or very cold ice may go unnoticed at the retail level. Consequently, based on the findings of this study, the use of freezer stored, cubed top ice cannot be recommended as a general practice.

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*PICKLE FIRMNESS AND THE DE OF PECTINS. . .*From page 140

1983), the immediate presence of Ca^{++} to block PME action would be required.

In summary, treatments that protected against softening of cucumber pickles during storage in brine also reduced the extent of demethylation of pectins. Firmness declined when the DE was reduced below the critical level of 12.3 ± 1.2. The mechanism responsible for retarding demethylation of pectins by $CaCl_2$, blanching or low temperature storage is unknown, but it probably is associated with hindering PME action. Excessive demethylation is believed to change the configuration of pectin macromolecules which contributes to loosening of middle lamella-cell wall components and softening.

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Effect of Lectins and the Mixing of Proteins on Rate of Protein Digestibility

LILIAN U. THOMPSON, ALAN V. TENEBAUM, and HOPPY HUI

ABSTRACT

The rate of digestibility of protein in raw bean extract (RBE), heat-treated bean extract (HBE), casein and bovine serum albumin (BSA) was determined. The pepsin and/or pancreatin hydrolysis of RBE which contains lectins or hemagglutinins was less than that of other proteins. Addition of lectins at the same concentration present in RBE decreased the rate of digestion of HBE, casein and BSA to levels close to that of RBE. In comparison with the respective single proteins, mixtures of RBE or HBE with casein have lower digestibilities while a mixture of casein and BSA, higher digestibilities. The results suggest that lectins can affect the activity of digestive enzymes and that mixing of proteins has an effect on digestibility which is unpredicted by amino acid composition.

INTRODUCTION

LEGUMES are good sources of protein, minerals, B-vitamins, dietary fiber and complex carbohydrates (Sgarbieri and Whitaker, 1982). They can lower blood lipids (Mathur et al., 1968; Jenkins et al., 1983), improve all aspects of diabetic control (Anderson and Ward, 1979), and their intakes are recommended by many health agencies (Health and Welfare Canada, 1977; Nuttall, 1979; Special Report Committee, 1981). However, legumes are also rich in many antinutrients such as trypsin inhibitors, tannins, phytates and hemagglutinins or lectins which can cause decreased digestibility of nutrients or toxicity when taken in large concentrations (Sgarbieri and Whitaker, 1982).

Lectins are proteins or glycoproteins which can bind to specific carbohydrate residues in cell membranes and are capable of agglutinating red blood cells (Lis and Sharon, 1973; Goldstein and Hayes, 1978). When taken in large amounts in free form or as raw beans, lectins can inhibit growth and cause death (Jaffe, 1980; Pusztai et al., 1982). The action of lectins is believed to be due to binding to intestinal mucosal cells, causing malfunction, disruption and lesion in the small intestine and consequently interference with the absorption of nutrients from the gut (Jaffe, 1980; Pusztai et al., 1982; Liener, 1974). Lectins and raw bean diets are nonlethal to germ-free animals and growth depression is significantly less, indicating that bacteria (normal gut flora) also play an essential part in their toxicity (Banwell et al., 1983; Jayne-Williams and Hewitt, 1972; Jayne-Williams and Burgess, 1974. Several workers (Pusztai et al., 1976; Wilson et al., 1980; King et al., 1980) have provided much of the evidence on the toxic effect of large intake of lectins using light microscopic studies of small intestines, nitrogen balance, microbiological and immunochemical studies.

The reduced availability of nutrients may also be due to the direct action of lectin on the digestive enzymes. However, very little work (Rea et al., 1985; Jindal et al., 1982) has been done to demonstrate this. Thus the objective of this study was to determine the effect of lectins, either in purified form or in bean extract, on the rate of protein digestibility by pepsin and pancreatin.

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MATERIALS & METHODS

AQUEOUS SUSPENSION (10%, w/v) of whole red kidney been flour was magnetically stirred for 30 min and centrifuged (1200 × *g*, 30 min) to obtain the raw bean extract (RBE). Heat treatment of RBE at 95°C for 20 min produced the heat-treated bean extract (HBE). The RBE and HBE were analyzed for protein using the micro-Kjeldal (AOAC, 1980) methods, phytic acid by the method of Latta and Eskin (1980), total polyphenol (tannins) by the method of Price and Butler (1977) as modified by Earp et al. (1981) and hemagglutinin activity using the method of Simpson et al. (1978) as described by Thompson et al. (1983). Concanavalin A (Pharmacia Fine Chemicals) was used as a standard in the hemagglutinin assay. Both RBE and HBE had 13.6 mg/mL protein, 0.70 mg/mL phytic acid and 0.19 mg/mL tannins. RBE had 1.6×10^6 hemagglutinin units/mL while HBE had nondetectable amounts.

The in-vitro pepsin proteolysis of RBE, HBE, casein (ICN Nutritional Biochemicals) and bovine serum albumin (BSA; Sigma Chemical Co.) as well as 50:50 protein mixture of casein and RBE, HBE or BSA was conducted with 110 mg protein substrate and 1.5 mg pepsin (from porcine stomach mucosa 1:60,000 2× crystallized; Sigma Chemical Co.) in 10 mL 0.1N HCl (pH 1.5) incubated at 37.5°C in a water bath. RBE and HBE were diluted with sufficient 0.1N HCl and adjusted to pH 1.5 to obtain 110 mg protein per 10 mL. After various digestion times, digests were analyzed for percent increase in amino group using the trinitrobenzene sulfonic acid method with glycine as the standard (Habeeb, 1966). Samples were also analyzed for percent protein precipitable in 5% trichloracetic acid (TCA) (Sgarbieri et al., 1981) with the precipitated protein determined using the Lowry et al. (1951) method. Blanks containing only the substrate and 0.1N HCl were treated under identical conditions.

The above method was also followed in the digestion of casein, HBE or BSA in the presence of lectin (concanavalin A) except that 8.1 mg lectin was added to the protein dispersion prior to the addition of pepsin. This amount of lectin has hemagglutinin activity (12.96×10^6HU) equal to that present in 8.1 mL RBE containing 110 mg protein. The lectin in 10 mL 0.1N HCl was also treated as above as an additional blank. Concanavalin A was used in this study since its effect on pepsin activity appears to be similar to that of red kidney bean lectin (unpublished data). It is also more readily available in purified form.

For in vitro pancreatin proteolysis, proteins were digested with pepsin for 90 min as above followed by the addition of 7.5 mL 0.1M phosphate buffer (pH 8.0) containing 4 mg pancreatin (Grade VI, from porcine pancreas; Sigma Chemical Co.) and incubation at 37.5°C for 3 hr. The digests were analyzed for percent increase in amino group and percent protein precipitable in 5% TCA as described above.

The reported values represent means of at least four determinations, each obtained as duplicate analyses of two replicate experiments. Unpaired t-test was used to determine significant differences between means. Only p values less than 0.10 were considered significant.

RESULTS & DISCUSSION

THE INITIAL EXPERIMENT was designed to determine the susceptibility of RBE, HBE and casein to proteolysis by pepsin. Casein was a control containing no lectins and other antinutrients while HBE was a control with the same composition as RBE except that the lectins and possibly trypsin inhibitors were inactivated.

The mean concentration of free amino group in RBE, HBE,

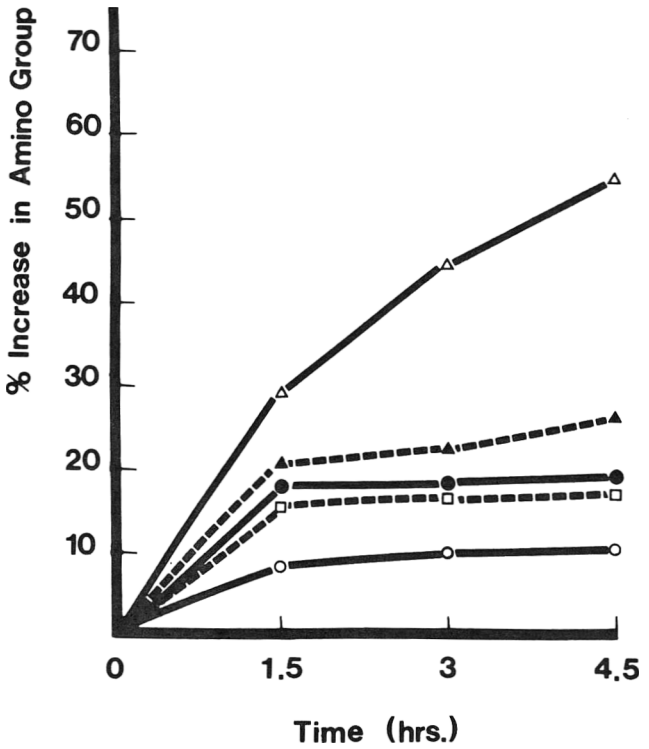

Fig. 1—Digestibility of: casein (△-△), raw bean extract (●-●), heat-treated bean extract (▲- - -▲), raw bean extract-casein mixture (○-○) and heat-treated bean extract-casein mixture (□- - -□) in the presence of pepsin. Mixtures were prepared in a 50:50 protein ratio.

Table 1—Mean concentration of free amino group in the protein solutions at zero hour of digestion

Sample	Fee amino groups[a] mg/mL
Casein	.71
Raw bean extract	1.22
Heat-treated bean extract	1.49
Albumin	1.53
Casein + raw bean extract	.90
Casein + heat-treated bean extract	1.01
Casein + lectin	.68
Heat-treated bean extract + lectin	1.00
Albumin + lectin	1.24
Albumin + casein	1.19

[a] Based on glycine as standard

casein and other samples tested at zero hour of digestion is provided in Table 1. Results expressed as percent increase in amino group (Fig. 1) showed that proteolysis of casein was significantly greater ($p<0.005$) than either RBE or HBE. This can be attributed either to the higher concentration of amino acids preferentially cleaved by pepsin in casein (West et al., 1970; Kanamori et al., 1982), to protease resistant sequences in bean protein (Boonvisut and Whitaker, 1976) or to the presence of antinutrients such as lectins, tannins and phytates in the bean extracts. HBE was digested to a significantly greater degree ($p<0.025$) than RBE although to a lesser extent ($p<0.025$) than casein. Since the phytic acid and tannin levels which can potentially decrease protein digestibility (Cheryan, 1980; Bressani and Elias, 1980) were similar in RBE and HBE, the negligible lectin level in HBE together with the denaturation of the proteins during heat treatment, may have been responsible for the increased HBE digestibility.

To determine whether the antinutrients in the RBE or HBE can decrease the digestibility of casein, the digestibility of their mixtures (50:50 protein ratio) was determined. While digestibilities lower than that of casein but higher than that of RBE or HBE were expected, significantly lower digestibilities of

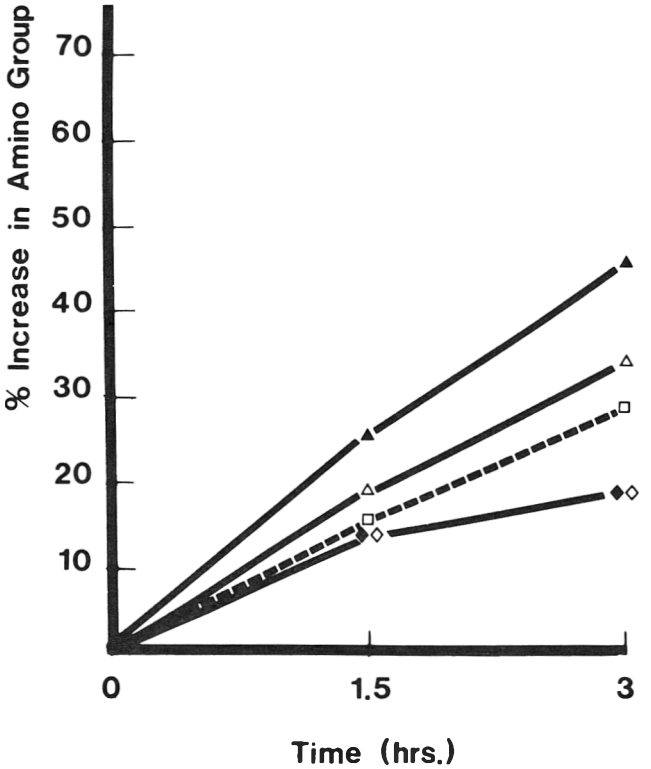

Fig. 2—Digestibility of: casein (▲-▲), casein plus lectin (△-△), heat-treated bean extract (□- - -□), heat-treated bean extract plus lectin (◇-◇), and raw bean extract (◆-◆) in the presence of pepsin.

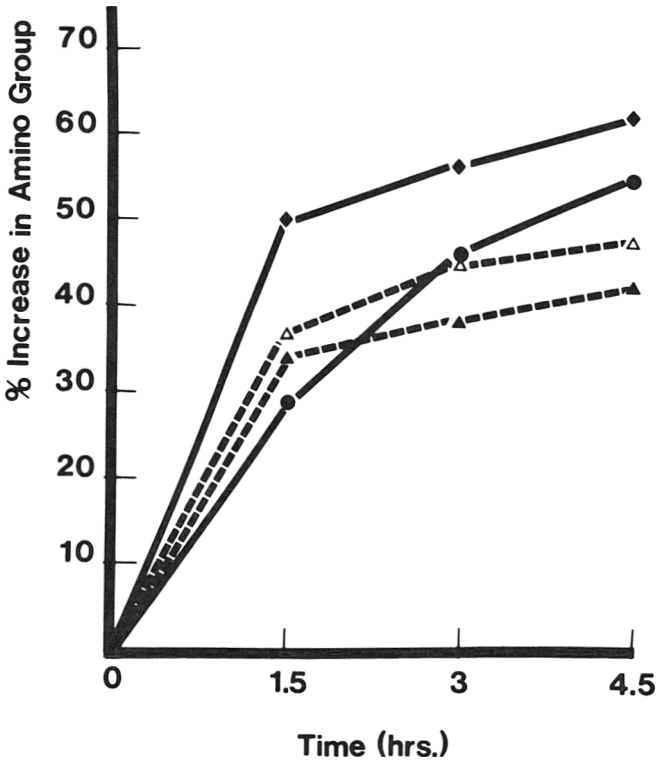

Fig. 3—Digestibility of: bovine serum albumin (BSA) (△- - -△), BSA plus lectin (▲-▲), casein (●-●) and 50:50 protein mixture of BSA and casein (◆- - -◆) in the presence of pepsin.

the casein-RBE ($p<0.005$) or casein-HBE mixtures ($p<0.01$) then either of the single proteins were observed (Fig. 1). This suggests that digestibility by pepsin is affected not only by antinutrients such as lectins but also the yet-to-be-determined

Table 2—Digestibility of proteins in the presence of pepsin and pancreatin

Sample	Increase in amino group %
Casein	86.4[a]
Casein + lectin	70.5[b]
Heat-treated bean extract	54.0[c]
Heat-treated bean extract + lectin	36.5[d]
Raw bean extract	33.9[d]

[a d] Means with different superscripts are significantly different ($p < 05$)

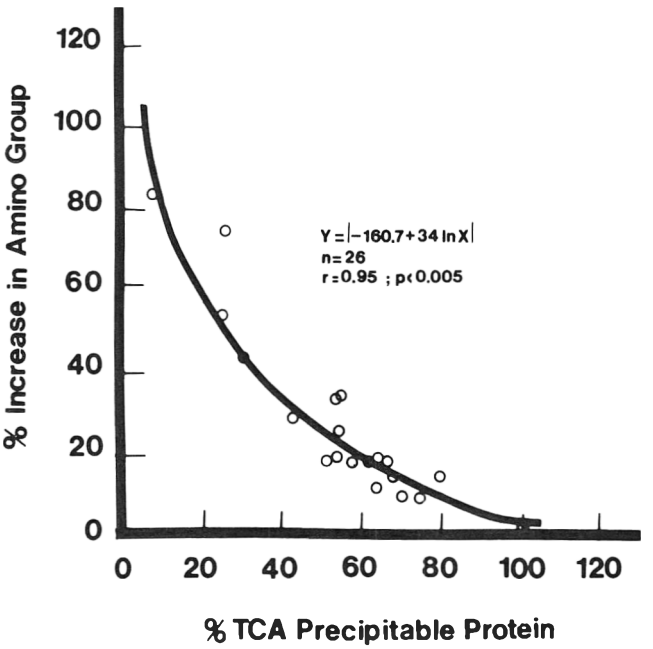

Fig. 4—Relationship between digestibility measured as % increase in amino group and as % trichloracetic acid (TCA) precipitable protein.

certain type(s) of protein-protein interactions which may occur at acidic pH.

To establish more clearly the role of lectin in lowering the digestibility of proteins, lectins were added to HBE or casein at hemagglutinin levels found in RBE. This resulted in proteolysis of CBE which did not differ significantly from that of RBE (Fig. 2) suggesting that lectin is a major factor in the pepsin digestibility of kidney bean proteins. Similarly, the addition of lectin to casein significantly reduced ($p<0.025$) the digestibility to levels approaching that of RBE. When lectin was added to another lectin-free protein (BSA) (Fig. 3), reduction in digestibility which did not differ significantly from the reduction observed with casein (Fig. 2) was also observed. All these results suggest a definite effect of lectins on pepsin activity.

Casein and HBE with or without added lectin were studied for rate of digestion after combined pepsin and pancreatin treatment. Trends obtained (Table 2) were similar to that obtained with pepsin treatment alone (Fig. 2). However, the digestibility differences between proteins were slightly greater indicating a lectin effect also on the activity of pancreatic enzymes.

A mixture (50:50 protein ratio) of casein and BSA was digested to explore the effect of mixing proteins in the absence of antinutrients. Using as a gauge the total amount of amino acids preferentially cleaved by pepsin, it might be expected that the BSA - casein mixture would be digested to a greater extent that casein but to a lesser extent than BSA. Casein has less concentration of amino acids preferentially cleaved by pepsin (West et al., 1970) than BSA. Results (Fig. 3) showed, however, a significantly higher hydrolysis of the mixture than either casein ($p<0.05$) or BSA ($p<0.005$).

The explanation for the synergistic effect on digestibility as seen with mixing of BSA and casein, and the antagonistic effect seen with mixing of bean extracts with casein is unclear. The study, nevertheless, suggests that mixing of proteins has effects on digestibility which can not be predicted by amino acid composition of the proteins.

The rate of protein digestion was monitored by measurement of percent increase in amino group and of percent protein precipitated by trichloracetic acid. Results in all experiments showed a very high and significant nonlinear correlation of 0.95 (Fig. 4) between the 2 methods.

In conclusion, this study showed that lectins or hemagglutinins can decrease the protein hydrolysis by pepsin and pancreatin. Hence, the low absorption of nutrients in the presence of lectins may not be due only to disruption of the intestinal mucosal cells by lectins but also due to the direct action of lectins on the digestive enzymes. This study also showed that mixing of proteins can increase or decrease the digestibility in a manner unpredicted by amino acid composition.

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Effect of Different Storage and Cooking Methods on Some Biochemical, Nutritional and Sensory Characteristics of Cowpea *(Vigna unguiculata* L. Walp)

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ABSTRACT

Cowpeas stored in a hermetic container, jute bag and nitrogen atmosphere at ambient (temperature 21–31°C, relative humidity 72–86%) for 6 months were evaluated for changes in proximate composition, nutritional and sensory qualities when cooked by different methods. Nitrogen storage effectively preserved the chemical composition of the cowpeas and maintained grain with better cooking characteristics than cowpeas stored in other systems. Cooking methods and the storage conditions influenced the cooking time and significantly affected the thiamine loss, rate of proteolysis, in-vitro protein digestibility and sensory qualities of the cowpeas. Cooking the stored unsoaked cowpeas with local rock salt to reduce cooking time had a deleterious effect on both the nutritional and sensory qualities of cowpeas.

INTRODUCTION

COWPEAS (*Vigna unguiculata* L. Walp) commonly called beans are an important grain legume in West Africa and many other tropical countries. Different local dishes prepared from cowpeas provide a significant amount of plant protein in the diet of many people in West Africa (Aykroyd and Doughty, 1965; Anon, 1981). Despite the nutritional value and increased production of cowpeas, consumption has not increased in rural and urban population like the cereal products. The long cooking times involved in the preparation of bean dishes have contributed to a decline in the consumption of cowpeas and other grain legumes in West African homes (Dovlo et al., 1976).

Storage of cowpeas in jute bags, metal drums, pots, and plastic bags with or without insecticides are common (Caswell, 1968). In view of the success reported for the storage of maize under nitrogen, it has been suggested that artificially induced controlled atmosphere involving the use of nitrogen could be adopted for cowpeas (Adesuyi et al., 1980).

Previous studies have shown that grain legumes such as black beans, chickpeas and pigeon peas when stored under adverse temperature and relative humidities similar to environmental conditions found in tropical climates (temp 21–31°C, RH 72–86%) for 10 wk or more, undergo biochemical changes which make them hard-to-cook (Muneta, 1964; Burr et al., 1968; Hall, 1970; Burr, 1973). Cowpeas purchased by individual households from the local markets in Africa have generally been stored for at least 10 wk or more. To soften the beans for consumption and to make them into convenience products which are palatable and digestible, the beans have to be cooked (Kakade and Evans, 1966). Cooking times for beans differ according to variety and processing treatments and may be quite long especially if the hard-to-cook condition had developed due to storage conditions (Kon, 1979).

Extended cooking of beans has reduced their consumption due to lack of convenience, fuel cost and nutritive value. Several processes have been described to prevent or reduce the development of the hard-to-cook phenomenon in beans (Rockland, 1964; Rockland et al., 1967, 1970; Burr et al., 1968; Molina et al., 1975, 1976; Kakadam et al., 1981). Presoaking of the legumes in water prior to cooking has been recommended and is quite popular in Asian culinary practices. However, there are no clear-cut relationships between the soaking characteristics of some legumes and their cooking rates (Burr et al., 1968; Youssef et al., 1982). But the most popular method of reducing the cooking time has been the cooking of the legume with alkali salts. The soaking and/or cooking of legumes with alkali salts cause loss of water, soluble vitamins and changes in bean morphology (Edijala, 1980; Miller et al., 1973; Nordstrom and Sistrunk, 1977; Varriano-Marston and Omana, 1979; Ankra and Dovlo, 1978).

In Nigeria and many parts of Africa, it is common practice to cook beans with a small amount of a local rock-salt (called 'Kaunwa or 'trona') which is believed to be a crude mixture of alkali potash and other salts. Its addition is known to reduce the cooking time of different bean varieties (Dovlo et al., 1976; Siegel and Fawcett, 1976).

Reports on the effects of traditional and improved storage methods and cooking practices on the quality of cowpeas are limited. This study was undertaken to evaluate the effect of storage methods on some biochemical and physical qualities of cowpeas. The effects of different cooking practices on thiamin retention, rate of proteolysis in-vitro digestibility and sensory quality of the cowpeas were also determined.

MATERIALS & METHODS

COWPEA (*Vigna unguiculata* L. Walp, Ife-brown variety), stored for 6 months in jute bags or metal drum (hermetic container) and under nitrogen, were obtained from the Nigerian Institute of Stored Products Research, Ibadan. Freshly harvested cowpeas of the same variety were obtained from the International Institute of Tropical Agriculture, Ibadan. All cowpeas were free from insect infestation, and no chemicals were used for preservation. In the inert atmosphere storage, the grains were kept under an artificially controlled atmosphere in a mini silo of 0.65 m^2 capacity. The atmosphere of the grain was replaced with nitrogen. The nitrogen atmosphere was maintained by continous introduction of a small amount of nitrogen (23.5 ± 7°C) to keep the controlled atmosphere inert. The jute bags, metal drum, and metal silo were kept in a room with natural air circulation at ambient conditions (21–31°C, RH 71–86%).

Chemical analysis

Samples of cowpeas were ground in a Wiley mill to pass a 40 mesh screen. The proximate composition for crude protein, ether extract, crude fiber, total ash, moisture was determined in duplicate by standard methods (AOAC, 1980). Nitrogen-free extract for total soluble carbohydrates was determined by difference.

Thiamin in the freshly harvested, stored and soaked or cooked cowpeas was determined by the thiochrome method (AOAC, 1980). The percent change in the thiamin was calculated. All determinations were made in duplicate.

Soaking treatment for physical characteristics

Fifty seeds (randomly selected) from the fresh or stored cowpeas were weighed and transfered to a 250 mL Erlenmeyer flask, and distilled water was added to 20 mL. The flask was lightly stoppered and left overnight (16 hr) at room temperature (23.5 ± 1°C). Prelim-

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Table 1—Proximate composition of freshly harvested and stored cowpeas (Vigna unguiculata L. Walp)[a]

Determinations	Freshly harvested	Hermetic container metal drum	Jute bag	Nitrogen atmosphere
Dry matter (%)	92.50	93.40	93.80	93.40
Crude Protein (N × 6.25) %	24.25[c]	21.12[b]	20.10[b]	24.10[c]
Crude fiber (%)	2.88	2.68	2.68	2.76
Ether extract (%)	3.35	3.25	3.10	3.42
Ash (%)	4.33	4.30	4.11	4.32
Total soluble carbohydrate (%)	56.41[c]	61.96[b]	63.73[b]	58.80[c]
Thiamin (mg/10g)	1.03[c]	0.75[b]	0.70[b]	1.10[c]

[a] Data represents the average of two determinations and are expressed on dry matter basis.
[a,b] Values significantly different from each other at 0.05% level of significance.

Table 2—Data on the soaking characteristics and cooking times of fresh and stored cowpeas[a]

Determinations	Freshly harvested	Hermetic container (metal drum)	Jute bag	Nitrogen atmosphere	Mean	S.D.
Hydration capacity (mg/seed)	17.2	16.2	14.8	17.8	16.30	1.09
Hydration index	182	160	150	181	168.25	15.84
Swelling capacity (mL)	192	175	176	190	183.25	8.96
		Cooking times (min)				
Cowpeas cooked without presoaking	120	130	145	132	129.25	11.35
Cowpeas 3 hr - presoaked	120	130	143	119	128.00	11.17
Cowpeas 6 hr - presoaked	90	110	120	93	103.25	14.22
Cowpeas 9 hr - presoaked	89	108	110	90	99.25	11.30
Cowpeas in pressure cooker	100	105	106	98	102.25	3.86
Cowpeas + 0.1% sodium chloride	125	135	140	133	133.25	6.24
Cowpeas + 0.3% sodium chloride	130	145	143	132	137.50	7.59
Cowpeas + 0.3% local rock salt	85	82	81	80	82.00	2.16
Cowpeas + 0.5% local rock salt	72	79	78	72	75.25	3.78
Cowpeas + 0.1% alkali potash	89	88	88	80	86.25	4.19
Cowpeas + 0.3% alkali potash	85	82	81	83	83.75	1.71
Cowpeas + 0.5% alkali potash	70	77	75	69	72.75	3.86
Cowpeas + citrate buffer pH 4.4	126	138	139	128	132.75	6.70
Cowpeas + citrate buffer pH 6.4	128	137	134	128	131.75	4.50
Cowpeas + alkali buffer 8.4	76	75	74	76	75.25	0.96
Cowpeas + alkali buffer pH 9.8	78	79	78	73	77.00	2.71

[a] Cooking times for fresh and nitrogen-stored cowpeas are significantly lower ($P<0.05$) than for cowpeas stored in jute bag or hermetic containers.

Table 3—Effect of cooking treatments on some nutritional quality characteristics of cowpeas[a]

Determinations		Freshly harvested	Hermetic container (metal drum)	Jute bag	Nitrogen atmosphere	Mean	S.D.
Thiamin retention (%)		(a) 50	51	50	51	50.50	0.25
		(b) 58	49	61	58	56.50	20.25
		(c) 33	32	25	34	31.00	12.50
		(d) 28	29	28	30	28.75	0.69
		(e) 68	67	59	68	65.50	14.25
Protein content (%)		(a) 22.1	21.2	21.5	22.0	21.70	0.14
		(b) 22.8	21.1	21.8	21.8	21.88	0.37
		(c) 21.8	20.8	20.3	21.0	20.98	0.29
		(d) 21.9	20.0	19.9	20.0	20.45	0.70
		(e) 21.1	20.0	19.6	19.0	19.94	0.59
In-vitro digestibility (%)		(a) 62	62	61	62	61.75	0.19
		(b) 66	63	62	64	63.75	2.19
		(c) 61	60	59	58	59.50	1.25
		(d) 52	50	48	45	48.75	6.69
		(e) 48	44	45	43	45.00	3.50

[a] Figures in row (a) refer to cowpeas cooked without presoaking; (b) cowpeas cooked but presoaked 6 hr; (c) cowpea cooked in pressure-cooker; (d) cowpea cooked with 0.3% local rock salt 'kaunwa'; (e) cowpea cooked with 0.3% sodium carbonate; (f) cowpea cooked with citrate buffer pH 6.4.

inary tests had indicated that the cowpeas had a maximum water imbibition at 16 hr. The beans were drained, blotted with an adsorbent paper to remove excess water and re-weighed. Hydration capacity per seed was recorded as weight after soaking minus weight before soaking.

Hydration index was determined as the ratio of the hydration capacity to the original seed weight. Some soaked seeds were re-weighed and transfered to a 200 mL measuring cylinder, and 100 mL water were added. Swelling capacity was defined as the increase in seed volume after hydration (16 hr). It was measured by water displacement as volume after soaking minus volume of the seeds before soaking. All determinations were made in triplicate.

Estimation of cooking time

Stored or freshly harvested cowpeas (control) soaked or unsoaked were cooked with six times their weight of distilled water or solutions of different levels of salts or buffers, pH 4.4, 6.4, 8.0, and 9.8. At intervals during cooking, samples were withdrawn and evaluated by a panel for uniform cooking and softness. Cooking time was determined by taking the mean time when there was no weight gain by the seeds and the cooked beans were judged to be soft to touch on pressing between the fingers (Zarkadas et al., 1965; Kumar et al., 1978). Ten independent replicates were made to obtain the mean cooking time for each treatment. Cowpeas were cooked separately with the following treatments: (a) in distilled water; (b) in distilled water after presoaking; (c) in pressure cooker (14.5 psi, 121°C); (d) in distilled water containing 0.1, 0.3, 0.5% w/v of the local rock salt (Kaunwa); (e) in sodium bicarbonate; (f) in sodium citrate buffer pH 4.4 or 6.4 or sodium bicarbonate buffer pH 8.4 or 9.8.

In vitro digestibility

The effect of the different cooking treatments on in vitro rate of proteolysis and digestibility was determined by the procedures adopted by Maga et al. (1973) and Saunders et al. (1973). Samples of the cooked cowpeas were air dried and ground to 80 mesh before the tests. Fifty milliliters of the suspended product (6.25 mg protein/mL) in glass-distilled water was mixed with 50 mg pepsin in 0.1N HCl. The pH of the resulting solution was adjusted to 7 with 0.1N HCl or NaOH and the mixture was incubated for 20 min. One milliliter of lyophillized trypsin at a concentration of 40 mg/mL was added to the solution which was stored at 37°C in a water bath. A rapid decline in

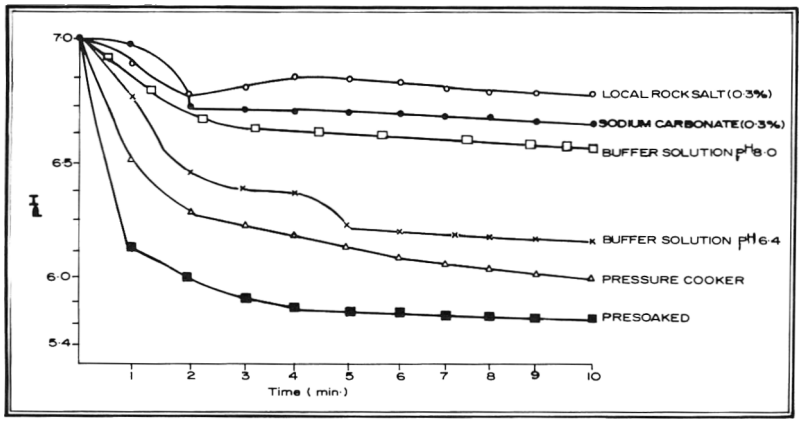

Fig. 1—Cooked cowpeas pepsin-trypsin hydrolysis rates.

Table 4—Sensory panel scores for the cowpeas cooked by different methods[a]

		Color	Flavor	Texture	Acceptability	Mean	S.D.
Fresh cowpeas	a	8	8	6	7	7.25**	0.96
	b	8	8	7	6	7.25	0.96
	c	3	4	4	4	3.75	0.50
	d	3	3	4	4	3.50	0.58
	e	6	5	4	3	4.50	1.29
	f	6	5	6	5	5.50**	0.58
Cowpeas stored in hermetic container	a	7	8	6	6	6.75**	0.96
	b	6	7	6	4	5.75**	1.26
	c	4	4	3	3	3.50	0.58
	d	3	4	3	3	3.25	0.50
	e	5	5	5	4	4.75	0.50
Cowpeas stored in jute bag	a	7	7	5	5	6.00**	1.16
	b	5	5	5	4	4.75	0.50
	c	3	3	3	3	3.00	0.00
	d	3	3	4	3	3.25	0.50
	e	5	3	3	3	3.50	1.00
	f	4	5	4	4	4.25	0.50
Cowpeas stored in N atmosphere	a	8	7	6	6	6.75**	0.96
	b	7	6	5	5	5.75**	0.96
	c	3	3	4	3	3.25	0.50
	d	3	3	3	3	3.00	0.00
	e	4	4	6	4	4.50	1.00
	f	5	5	5	3	5.00	0.00

[a] Scores are based on a 9-point hedonic scale where 1 = dislike extremely, 9 = like extremely. Figures in row (a) are for the cowpeas cooked unsoaked; row (b) cowpeas 6 hr presoaked prior to cooking; row (c) cowpeas cooked with 0.3% local rock salt; row (d) cowpeas cooked with 0.3% alkali potash; row (e) cowpeas cooked with pressure cooker; row (f) cowpeas cooked in citrate buffer pH 6.4. Figures with the same asteriks are statistically significant at 5% level.

pH occurred immediately. This was caused by the freeing of the amino and carboxylic groups from the protein chain by the proteolytic enzymes. The pH drop was recorded automatically over a 10 min period using a recording pH meter. After allowing the reaction to continue for 16 hr, the insoluble solids were separated by centrifugation, washed with water (3 × 30 mL), filtered through a 1.2 filter (Millipore), air dried, weighed and analyzed for nitrogen by the micro-Kjeldahl method (AOAC, 1980). The calculation was as follows:-

$$\text{Digestibility (\%)} = \frac{\text{N in the cooked cowpeas} - \text{N in undigested fraction}}{\text{Nitrogen in the cooked cowpeas}} \times 100$$

Sensory evaluation

The cooked cowpeas were coded and submitted to a 10 member panel for evaluation. The members were 5 men and 5 women from the university community who were familiar with bean products. Panelists were asked to score the products on a 9-point hedonic scale where 9 = like extremely and 1 = dislike extremely for the quality attributes of color and texture as felt between the fingers, bean-wholeness, flavor and acceptability. The data from three panel sessions were averaged and analyzed statistically for significance using the multiple range test (Steele and Torrie, 1960).

RESULTS & DISCUSSION

THE PROXIMATE COMPOSITION of the fresh and stored cowpeas is shown in Table 1. There was a significant decrease in the levels of protein in the cowpeas stored in jute bags and metal drum (hermetic container) compared to the fresh cowpeas (control) and beans stored under nitrogen. No changes were obtained in crude fiber and ash. Significant ($P < 0.05$) losses of thiamin occurred in cowpeas stored in the jute bags and metal drum. Nitrogen-stored cowpeas maintained a chemical composition similar to the freshly harvested cowpeas. The decrease in protein, thiamin and soluble carbohydrates of the cowpeas stored in jute bags and metal drums was similar to the losses observed in chickpeas and lima beans stored under similar conditions (Burr, 1973). These data emphasized the advantage of using nitrogen over other methods for storing cereals and other grain legumes in maintaining good quality (Adesuyi et al., 1980).

Data on the physical changes in the soaked cowpeas and cooking times under different treatments are presented in Table 2. Cowpeas stored in nitrogen behaved like the freshly harvested grains and had a significantly better ($P<0.05$) hydration capacity and swelling capacity than cowpeas stored in the jute bags and metal drum (hermetic container). Cowpeas stored under these two treatments also showed shorter cooking times than cowpeas stored in jute bags or metal drum.

Although soaking and cooking rates are separate phenomena which are not necessarily correlated, evidence indicates that hydration capacity is related to cooking times of chickpea and black beans (Molina et al., 1976; Yousseff et al., 1982; Williams et al., 1983). Significant reduction in the cooking times of cowpeas which were presoaked, cooked in a pressure cooker or cooked in water containing rock salt or alkali buffer confirmed literature findings and observations made by many African families (Feldberg et al., 1956; Muneta, 1964; Burr et al., 1968; Hoff and Nelson, 1966; Rockland et al., 1967; Meolina, 1976; Kakadam et al., 1981; Ankra and Dovlo, 1978).

Soaking beans prior to cooking is not a common practice in West Africa (Siegel and Fawcett, 1976), although it is common in other cultures. However, presoaking stored cowpeas for at least 6 hr reduced cooking time. Longer soaking times had no beneficial effect and may lead to loss of nutrients prior to cooking (Onayemi et al., 1976). Storage conditions did not play a major role in cooking time when alkali salts were present except that rehydration characteristics were affected. Tables 3 and 4 show the effects of the different cooking treatments on the nutritional and sensory quality of the cowpeas. Addition of sodium carbonate or local rock salt not exceeding 0.3% is reported because salt levels beyond 0.3% caused the cooked

cowpeas to have an unacceptable dark color which the panelists rejected.

Cowpeas cooked with sodium carbonate, rock salt, sodium chloride, citrate or alkali buffer had chemical and sensory quality characteristics different from the control. Thiamin content in cowpeas presoaked 6 hr or cooked in the pressure cooker was 40–50% lower than in the original uncooked cowpeas or cowpeas cooked to a soft texture in the traditional manner. Soaking cowpeas beyond 6 hr for ordinary household consumption did not have any beneficial effect on thiamin retention (Onayemi et al., 1976), and the cooked product had a gummy texture.

Significant reductions in the thiamin ($P<0.05$) of cowpeas occurred on addition of alkali salts to the cooking medium. No loss of thiamin was observed in cowpeas cooked with sodium chloride or in citrate buffer pH 4.4 or 6.4. This observation is in accordance with the stability of thiamin in acid conditions (Farrer, 1955). Loss of thiamin in cooked beans was affected more by the cooking treatment than by the previous storage history.

The in vitro rate of proteolysis, which is an indicator of the digestibility of the cooked cowpeas, is shown in Fig. 1. Cowpeas cooked either with sodium bicarbonate or the rock salt had reduced digestibility compared to cowpeas cooked by other methods to the same degree of softness. Decreases in digestibility were proportional to the increase in the concentration of the salts and thus indicated that these salts probably caused some changes in the cowpea proteins as reported (Sefah-Dedeh et al., 1978). Table 3 shows data on gross protein and thiamin levels and in-vitro-digestibility of cooked cowpeas and indicated that these alkali salts reduced the nutritive value of the bean products.

Cowpeas cooked after presoaking, without alkali salt, or in a pressure cooker had better digestibility than cowpeas cooked at atmospheric condition or with alkali salts (local rock salt "kaunwa" or alkali salt). The nitrogen stored cowpeas had higher digestibility values than cowpeas stored in other systems, possibly due to the reduction in cooking time of nitrogen-stored cowpeas.

Taste panel scores for the fresh (control) cowpeas and stored cowpeas cooked under different conditions are presented in Table 4. The type and level of the salts added to the cowpeas as well as the presoaking times altered the sensory quality characteristics of the cooked cowpeas. The data represent the average of three panel tests and were for cowpeas cooked with alkali and the local rock salt at levels not exceeding 0.3% (w/v). Beyond this level of salt, the products were rejected by the panelists for their poor color and texture. Cowpeas cooked with 0.2% sodium chloride or in citrate buffer had good flavor, less splits and retained grain wholeness compared to cowpeas cooked after presoaking (6 or 9 hr) or in a pressure cooker. Addition of 0.5% alkali salt or rock-salt to the cowpeas resulted in cooked cowpeas with a poor color, sharp flavor, considerable splits and mushy texture with no characteristic wholeness when the scores for those attributes were compared to cowpeas cooked without these salts. Consumers of beans preferred products which were not darkened by these salts and were not sticky or gummy.

Cowpeas stored in nitrogen presoaked or unsoaked and cooked without the addition of the rock-salt or the alkali salt for a period not exceeding 6 hr were rated as high as the fresh cowpeas in flavor, texture and acceptability compared to cooked cowpeas stored in the jute bags or metal silo.

Nitrogen storage of cowpeas involves a high initial capital cost when compared to storage of cowpeas using simpler systems. The use of nitrogen for storing grains could be adopted at the community level in the major cowpea producing regions as part of the overall program for reducing food loss and preventing the misuse of chemical preservation methods. Nitrogen storage maintains the good quality attributes of cowpeas, including nutritional and water imbibition properties. It also eliminates the risk of residual effects of chemical preservatives. To develop a convenience canned product for West African consumers, processors could store a good quantity of cowpeas in nitrogen or other inert atmospheres. If the cooking times are to be reduced by the addition of alkali salts or local rock-salts, salt-levels should not exceed 0.3% in order for the product to be acceptable and to have good nutritional qualities.

CONCLUSION

NITROGEN STORAGE of cowpeas for 6 months preserved the thiamin of the cowpeas and also prevented the changes that lead to the hardshell found in cowpeas stored in jute bags or hermetic containers (metal drum).

Soaking characteristics were affected by storage conditions. The nutritional and sensory quality characteristics of the cooked cowpeas were affected by the method of cooking and the type and amount of alkali salt added. For cowpeas stored in jute bags or in the metal silo, which are conducive to hardshell, presoaking for a period up to 6 hr reduced the cooking time of the beans, if a pressure cooker was unavailable or very expensive for a household.

This treatment did not reduce the thiamin nor the digestibility of the cooked cowpea when compared to the traditional practice of cooking the cowpea at atmospheric pressure or with alkali salt or local rock-salt to reduce the cooking time and save fuel cost. The practice of using kaunwa or local rock salt must be discontinued because of its deleterious effect on the nutritive value of the cooked beans. Processors of convenience cowpea products can now select a storage system and cooking practice that would enable them to maintain continuous operation and production of a nutritious bean product.

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Influence of Reducing Sugars and Amino Acids in the Color Development of Fried Potatoes

G. MARQUEZ and M.C. AÑON

ABSTRACT

Color development during potato frying was studied. Increments of reducing sugars led to increase in color of fried potatoes. For L (luminosity) between 60–51, corresponding to a reducing sugar content of 120–140 mg %, an acceptable color was obtained. Both amino acids and reducing sugars participated in the color development of potato during frying, with the amount of reducing sugars being the limiting factor. Fructose yielded the highest browning followed by glucose. Sucrose addition caused practically no change in the final color of the fried potato. At low content of reducing sugars the reaction followed first-order kinetics, the activation energy, Ea, being equal to 31 Kcal mol^{-1}.

INTRODUCTION

PREVIOUS STUDIES have shown various factors to be influential in determining the color of potato chips and french fries (Habib and Brown, 1956, 1957; Lyman and MacKay, 1961; Shippers, 1975; Miller et al., 1975; Gould et al., 1979). It has been suggested that the color depends upon the formation of brown pigments during the frying of the potato slices in oil, resulting from the reaction between sugars and amino acids. The reducing sugar content of the tuber is affected by storage temperature and/or time in storage (Watada and Kunkel, 1955; Isherwood, 1973, 1976; Iritani and Weller, 1977; Dwelle and Stallknecht, 1978). Storage of potatoes at low temperatures (below 7°C) causes accumulation of reducing sugars, with much darker chips being produced from tubers with higher content of sugars. The complexities of the color problem are not well understood although it has been extensively studied. The main objective of this research was to accumulate more data on color development during potato frying in order to define the best conditions for producing frozen French fries.

MATERIALS & METHODS

Material

Tubers of Kennebec cultivar were grown at the National Institute of Agricultural Technological station at Balcarce (INTA Balcarce-Argentina) during the growing seasons of 1982 and 1983.

Storage conditions and sampling

After harvest, tubers were graded, randomized and separated into two lots. Parts of each lot were stored in cold rooms at 3°C and 10°C to simulate industrial conditions. Samples were taken for periodic measurements of dry matter, sugar and amino acid as follows: four replicate samples (five tubers per replication) were washed, peeled and cut into slices. Portions were obtained from cortical and medullary tissue. The portions were rinsed in distilled water, drained, and for each replication, randomly sampled for dry matter, sugar and amino acid analyses.

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Dry matter

Dry matter was obtained by weighing 10-g samples in tared aluminum paper dishes and drying at 60°C to constant weight (AOAC, 1970).

Extraction of sugars and amino acids

Medullary and cortical tissue samples were cut into small pieces and the sugars and amino acids extracted from 10 g samples homogenized in a Sorvall Onnimixer for 2–3 min with 40 mL of boiling 96% ethanol. The ethanolic extracts were filtered, and the sediments were washed twice with 50-mL portions of 80% ethanol. The clear solutions were made up to 150 mL with 80% ethanol. At the time of use the ethanol was removed in a Rotavapor-M Buchi HB-140 (Switzerland).

Total and reducing sugars determination

The total and reducing sugars were determined according to the method described by Cronin and Smith (1979) and by thin layer chromatography (TLC). In the latter case 2 and 5 µL of the aqueous sugar mixture were spotted on silica gel 60 plates. The chromatoplates were developed with 1-propanol-ethyl acetate-water (70:20:10) v/v in the ascending direction at 20°C. Then the plates were removed, dried in air and developed again. After drying the sugar spots were detected by spraying the chromatoplate with anisaldehyde-sulfuric acid solution (0.5 mL anisaldehyde-9 mL 95% ethanol-0.5 mL concentrated sulfuric acid-0.1 mL glacial acetic acid) followed by heating at 100°C for 10 min. The plates were scanned in a dual wavelength TLC Scanner CS-910 (Shimadzu, Kyoto, Japan) (sample wavelength 610 nm and reference wavelength 410 nm) attached to C-R 1A Chromatopac Shimadzu integrator.

Amino acids determination

Free amino acids were derivatized with dansyl chloride before placing them on an HPLC column for determination (Gray, 1967). The Waters Associates (Milford, MA) equipment used consisted of an M 6000 A Solvent Delivery System, a Model 660 Solvent Programmer, and a Model 450 variable-wavelength detector at 254 nm. The column used was 3.9 mm × 30 cm µBondapak C18 (Waters Associates). The mobile phase was: Solvent A: CH_3CN/0.02N acetic acid (10/90); Solvent B: CH_3CN/0.02N acetic acid (90/10); isocratic for 5 min; then linear gradient 10% B-55% B over 45 min; flow rate: 1.5 mL min^{-1}.

Standard amino acids were obtained from Sigma Chemical Company, (St. Louis, MO).

Table 1—Effect of addition of soluble sugars on color development

Glucose (mg %)	Fructose (mg %)	Sucrose (mg %)	Luminosity (L)
476	980	206	30
995	405	215	35
184	246	900	50-49

Table 2—Soluble sugar contents before and after frying

	Glucose (mg %)	*Fructose (mg %)*	*Sucrose (mg %)*
Raw slice	*204.3*	*310.7*	*206.3*
Fried slice	*319.0*	*419.5*	*226.0*
Crust	*147.2*	*110.4*	*187.3*

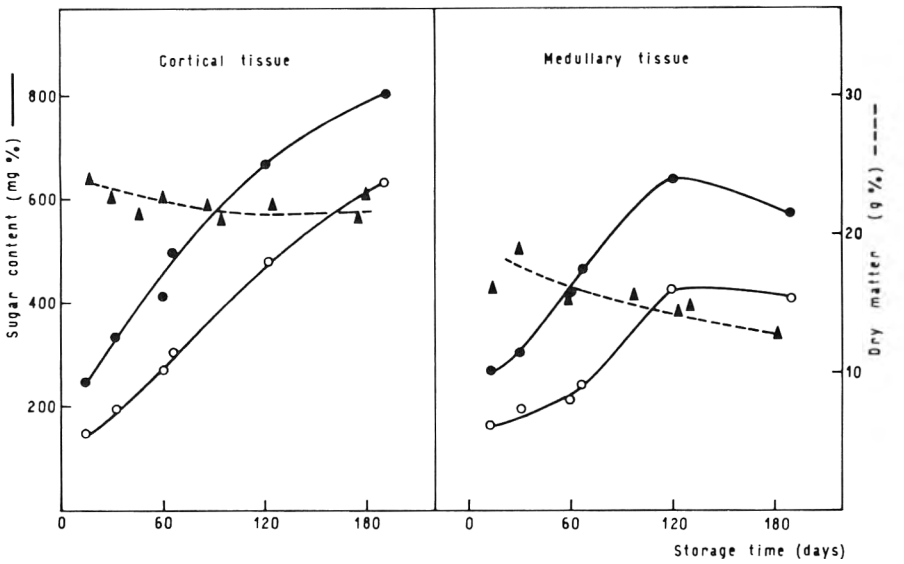

Fig. 1—Changes in reducing and total soluble sugars and dry matter of Kennebec potatoes during storage at 3°C (○) reducing sugars; (●) total soluble sugars.

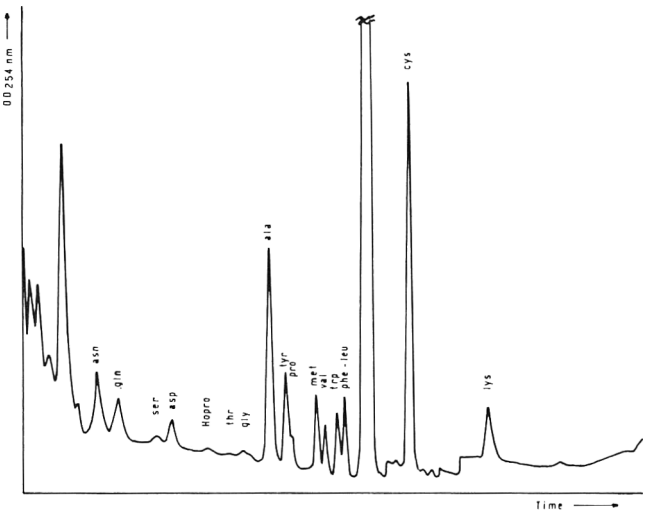

Fig. 2—Chromatogram of amino acids of raw potato extract.

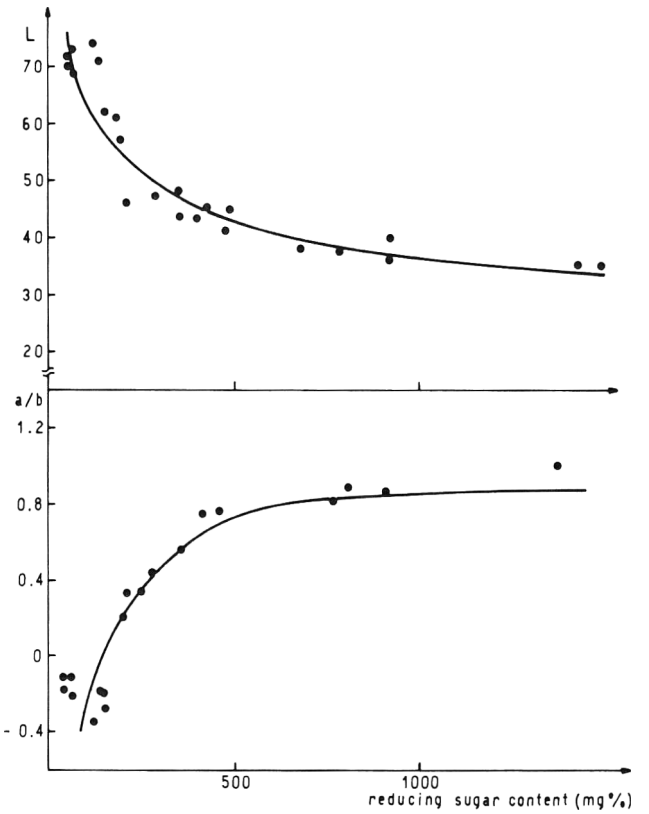

Fig. 3—Effect of reducing sugar additions on color development of fried potatoes: L, luminosity; a/b, chromaticity ratio.

Frying conditions

At different times of storage, slices (5 cm diameter, 0.4 cm thick) were taken from tubers. The slices were fried in sunflower seed oil at 180°C for 3 min. The fried potatoes were drained and their color was immediately measured in a HunterLab color/difference meter D25-3 (Fairfax, VA).

RESULTS & DISCUSSION

Metabolic changes during storage

Typical changes in dry matter, reducing and total soluble sugars of Kennebec potatoes stored at 3°C are shown in Fig. 1. Dry matter was significantly lower in the pith area than in cortical tissue, while no significant differences were detected between these areas in respect to sugar content.

The ratio of reducing sugars/total soluble sugars increased during storage from 55% at the beginning of storage, to 80% after 5 months at low temperature. The analysis of the tuber sugars during this period showed a great increase in fructose and glucose (fourfold) and a smaller increase of sucrose (by 50%). Since glucose was lower than fructose, these reducing sugars did not accumulate entirely as a result of the hydrolysis of sucrose. Correlation between the level of sucrose and the level of reducing sugars was poor (results are not shown). Reducing sugars remained practically constant during 6 or 7 days and then increased steadily.

At 10°C reducing and total soluble sugars did not change appreciably during the first week of storage and then decreased when tuber sprouting approached.

Seventeen free amino acids were identified in the tuber, but there were no significant differences in their levels during storage at either of the temperatures employed (Fig. 2).

Color development

The effects of storage on the color of French fries were observed. The color quality of the fried potato slices produced from tuber stored at low temperature (3°C) was markedly decreased. The discoloration was due to accumulation of reducing sugars. Luminosity (L parameter) decreased and chromaticity (ratio between parameters a and b) increased with increase of

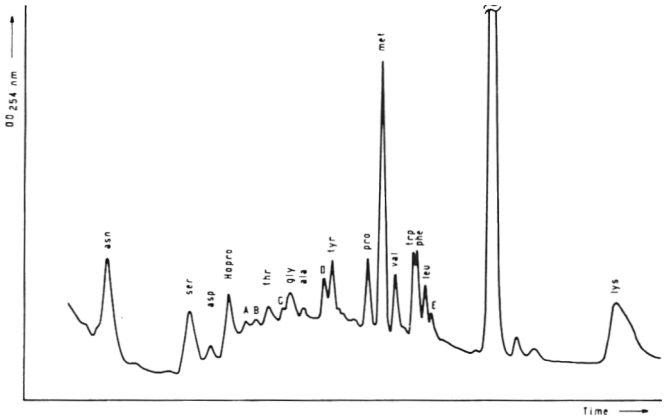

Fig. 4—Chromatogram of amino acids of extract of the crust of fried potato. The capital letters identify the peaks which could not be associated with amino acids.

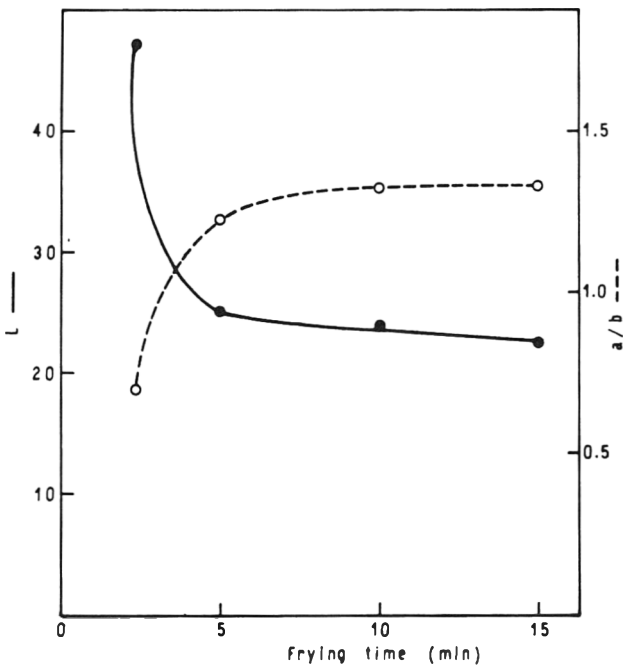

Fig. 5—Color development as a function of frying time.

reducing sugars. The experimental data of Fig. 3 were subjected to a regression analysis (Marquardt method) and the following equations were obtained:

$$L = D\,x^{-1/E} \quad (1)$$

$$x = A\,e^{a/b} + B\,e^{C.a/b} \quad (2)$$

where L is the luminosity; a/b is the chromaticity ratio and x is the reducing sugar concentration. The parameter values in Eq. (1) were D = 197.3 and E = 4.11, and in Eq (2), A = 107.9; B = 7.32 and C = 5.2.

In terms of L an acceptable color was obtained in the range 60–51, corresponding to a reducing sugar content of 120–240 mg %. Below this L value the color became very dark and above it too light, both unacceptable. In terms of the chromaticity ratio the limit of color acceptability was −0.2 to 0.35.

In order to determine only the effect of storage at low temperature, ΔL, Δa and Δb were calculated (by subtracting from each value the one corresponding to zero time); and the color difference according to the Hunter scale

$$[\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}] \quad (3)$$

was calculated. A strong increase of ΔE with storage time was observed, and it correlated with the reducing sugars increase (results are not shown).

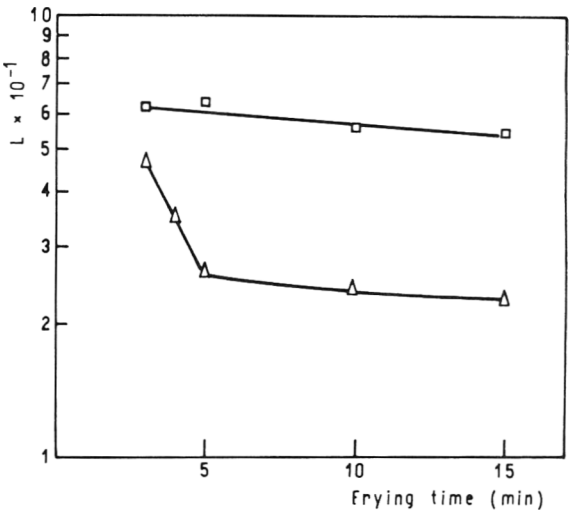

Fig. 6—Logarithm of L as a function of frying time (□) low concentration of reducing sugars; (△) high concentration of reducing sugars.

As can be seen from the data of Table 1, addition of fructose increased the final color of the French fries more than an equal addition of glucose. Similar increase of sucrose did not modify the final color appreciably. These results are in agreement with previous studies carried out in model systems (Ashoor and Zent, 1984).

Soluble sugars and free amino acids were determined in the pulp (inner part) and in the crust of the potato slice after frying. Table 2 shows the decrease detected in reducing sugars in the crust. Sucrose content did not change upon frying. In the pulp no changes were detected; there was, however, an increase in the total amount of reducing sugars (probably owing to starch hydrolysis).

Analysis of a water extract of the crust by HPLC showed a decrease in the amino acids. The chromatograms presented a series of peaks (Fig. 4 identified by capital letters) which could not be associated with amino acids (to identify them a simpler experimental system is being developed). Amadori compounds formed by reaction of sugar and amino acids has been isolated from browned freeze-dried apricots (Anet and Reynolds, 1957); tomato powder (Eichner and Ciner-Doruk, 1981) and potato flakes (Sapers et al., 1972).

These results showed the participation of both amino acids and reducing sugars on color development of potato during frying. The limiting factor of the reaction could be reducing sugar since free amino acids content did not significantly vary with time or temperature of storage.

Influence of frying conditions on color development

Figure 5 shows the reaction kinetics of color development at high concentration of reducing sugars. A strong color change was produced between 3 and 5 min frying; later, a saturation state was reached. The linear portion of the semilog plot suggested pseudo-first-order kinetics during the early stage of the reaction (Fig. 6). At low content of reducing sugars the color development followed first-order kinetics as indicated by the straight line obtained when plotting the logarithm of L vs time of frying (Fig. 6).

The effect of frying temperature at low sugar content on the color development is shown in an Arrhenius plot (Fig. 7), which allows the calculation of activation energy. The computed value was 31 Kcal mol^{-1}, equivalent to those reported for other nonenzymic browning reactions (Karel, 1982; Lee et al., 1984).

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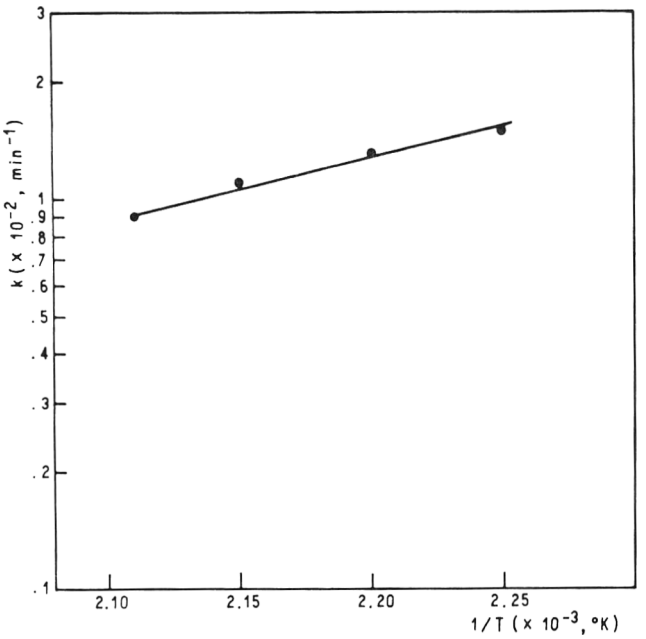

Fig. 7—Arrhenius plot for the browning reaction at low sugar content.

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Some Chemical Factors Affecting the Quality of Processed Yam

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ABSTRACT

Three species of yams (1) white (2) water, and (3) yellow were studied. Each was divided into three sections: head (stem end), middle and tail-and examined for quality characteristics which are important in the utilization of yam for convenience foods. The characteristics studied were susceptibility to discoloration and chemical composition including nutrients such as ascorbic acid, nitrogen constituents, total lipid and polyphenols. The polyphenol content was positively correlated with discoloration and was affected by storage time as well as by the section of yam utilized. The concentration of polyphenols was highest in the head region, which exhibited the greatest discoloration. The middle section was most suitable in preparating light-colored yam products since it was lowest in phenols and exhibited the least discoloration.

INTRODUCTION

THE YAM (*genus Dioscorea*) is an important food crop in the diet of many West Africans. It is second to cassava as a carbohydrate source in terms of consumption and economic value. Nutritionally, yam is a better source of protein and ascorbic acid than cassava (Coursey, 1967; Rasper and MacGregor, 1969; Oke, 1972; Martin and Thompson, 1971). Martin and Sadik (1977) described the significance of yams to the economic, cultural and social life of West Africans and the potential contribution that some yam species could make to the food resources of some tropical countries. The use of yam in livestock feed and for starch in pharmaceutical preparations is limited by the high cost of producing yam tubers, storage problems and the presence of mucilagenous substances in the starch when compared to cassava (Coursey, 1967). Only a few varieties are used for food preparation.

In spite of the improvement in yield, tuber size, shape and storage problems of yam, little is known about the effect of the chemical factors on the quality of processed yam products. In the potato tubers and other vegetables, differences in chemical composition have been shown to affect the selection or suitability of the tubers for certain products, and this in turn affects the sensory quality of the products made from the tubers (Schwimmer and Burr, 1967).

Rasper and Coursey (1967) described the rheological and microscopic properties of starch from different yam varieties and suggested that these might be related to the textural quality of yam products. Rodriquez-Soza et al. (1972) evaluated the suitability of some yam varieties for the production of yam flakes. Variability in the discoloration of the raw yam tuber constitutes a major drawback of some species as foods. Polyphenolic substances have been implicated in the discoloration often observed in yam tubers (Martin et al. 1974; Martin and Ruberte, 1976). Yams vary in their susceptibility to discoloration and this variability constitutes a major problem in the utilization of some yam species as food. The taste and flavor of yam tubers also vary depending on the species, maturity and length of storage. The bitterness of yellow yam (*Dioscorea cayanensis*) has been attributed to the presence of water-soluble phenolic substances (Martin and Ruberte, 1976). The effects of the polyphenols, carotenoids and total lipids on the color and flavor qualities of potato products have been reported (Mondy et al., 1967, 1971; Reeve et al., 1969; Matthew and Parpia, 1971).

During harvest, consumption of pounded yam is quite common (pounded yam is usually prepared by pounding cooked white yam or occasionally yellow yam with a wooded pestle and mortar until a dough-like texture is obtained). When there is surplus harvest, the yams, peeled or unpeeled are cut into 2.5 cm slices, parboiled in water at 60°C for 30 min and then sundried. During sun-drying the yam pieces often turn brown due to phenoloxidase activity causing flavor deterioration. The dried slices are either sold as such or milled into flour. For consumption the flour will be reconstituted with stirring while cooking at which time it turns black in color and pasty in texture.

These products have varying degrees of acceptability and preference because of their flavor and color. Some yam varieties are highly valued while others which are higher in dry matter and nutrients are not fully utilized. There are not many published reports on the influence of the chemical composition of yams on the quality of the various products.

This investigation was undertaken to determine the relationship of polyphenol content to discoloration of yam products. The effects of variations in the chemical constituents within the tuber and length of storage on the yam products were also studied.

MATERIALS & METHODS

Sources of material

Three species were used in this investigation: (1) white yam (*Dioscorea rotundata*); (2) yellow yam (*D. cayanensis*); and (3) water yam (*D. alata*). Mature yams were harvested on farms located within 50 km radius of the University of Ife. The tubers were carefully handled to avoid bruises or damage by mold and divided into two lots. One lot of each yam species was stored for three months under conditions simulating local storage practice which consists in keeping the tubers on a raised platform under a shed and covering the tubers with leaves to provide ambient air circulation (23–31°C, relative humidity 72–86%).

Randomly selected tubers from the freshly harvested and stored lots were hand peeled to remove 3 mm of the outer portion of the tuber. Peeling was done on each occasion prior to proximate analysis for moisture, starch, crude fiber, protein and ash by standard methods (AOAC, 1970). Each peeled tuber was divided into head, middle and tail sections.

Determination of discoloration

A 2 mm slice of each tuber section was removed with a stainless steel blade and placed immediately in an atmosphere of chloroform and visually compared for the extent of discoloration. At 20 min intervals a panel of 6 experienced judges, selected on the basis of their ability to detect color differences, evaluated the intensity of discoloration using a 5 point scale where 1 = slightly discolored, 3 = moderately discolored and 5 = very discolored (Mondy et al., 1967). The tests were replicated four times and performed on the fresh and stored tubers. Data were analyzed for significance using the rank order test (Kramer, 1960).

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Determination of polyphenols

Ten tubers of each species were randomly selected, peeled, washed and divided into head, middle and tail sections. A 50g portion of each section was blended with 150 mL of 95% ethanol for 5 min. Thirty-five milliliters of the filtered extract were used for the phenol determination (Rosenblatt and Peluso, 1941) using tannic acid as the standard. Since this method is not specific for phenolics and also involves ascorbic acid, corrections were made for ascorbic acid. Duplicate determinations were run on each section from the fresh and stored tubers.

Determinations of ascorbic acid

Ascorbic acid of the sections of tubers from the freshly harvested or stored yams was determined in duplicate on metaphosphoric acid extracts by titration with 2,6-dichlorophenolindophenol reagent (AOAC, 1970).

Determination of total lipids

Total lipids were extracted from sections of the tubers with chloroform-methanol mixture (2:1) under reflux. Duplicate determinations were made on the fresh and stored tubers, and the average results of the total lipids content are reported.

Determination of total nitrogen

Sections from the fresh and stored peeled tubers of each species were analyzed in duplicate for total nitrogen by the micro-Kjeldahl method (AOAC, 1970).

Preparation of yam products

Fresh and stored yams of each species were processed into flour by the traditional method and instant pounded flour by a newer improved method. For the traditional flour, yams were peeled, washed, cut into 13 mm slices, heated in water (60°C for 3 min), oven-dried and milled (20 mesh). For instant pounded yam flour, selected yams were peeled, washed, diced to cubes (9.5 mm), dipped in sodium metabisulfite solution (250 ppm) for 10 min and steam-blanched for 10 min. After rinsing with water, the air-dried cubes (70°C in a cabinet dryer) were milled with a Hammer mill to pass through a 20 mesh screen. The instant pounded yam flour or yam flour was made with either the head, middle or tail sections or the whole portion of each peeled yam from each of the three species. The instant pounded yam flour reconstitutes to the same texture as the traditionally prepared pounded yam which is usually prepared by pounding cooked yam in a wooden pestle and mortar until a dough-like texture is obtained.

The two yam products made from either the whole tuber or sections of the tuber of each species were reconstituted separately by stirring a measured weight of material into boiled water (in the ratio of 1 part product to 3 parts water) as it is traditionally prepared. The reconstituted products was cooked until the desired texture was obtained.

The reconstituted yam products made from the stored tubers were evaluated by a five member panel for the attributes of color, flavor and acceptability using a 9 point hedonic scale where 1 = dislike extremely and 9 = like extremely. Data on the panel scores were converted to ranks and analyzed for significance using a method reported by Kramer (1960).

RESULTS & DISCUSSION

THE EXTENT of tuber discoloration in relation to polyphenol content of the yam species, section of the tuber and storage is depicted in Fig. 1. There were significant differences ($P<0.05$) in discoloration among the tubers and within the tuber regions. The intensity of discoloration varied from 5 for the water yam to 2 for the white yam and in all the tubers generally, the head section (stem end) had a higher degree of discoloration (value of 4) compared to the middle section. Water yam (*D. alata*) showed the highest susceptibility to discoloration followed by yellow yam while the white yam was the least discolored. The discoloration in the yam section followed a general pattern in decreasing order: the head section was the most susceptible to discoloration; the middle section showed the least. The stored tubers were generally more susceptible to discoloration than the fresh tubers with the head and tail regions showing significantly more discoloration than the fresh tubers with the head and tail regions showing significantly more discoloration than the other sections. Similar observations of varietal differences in yam tuber discoloration have been previously reported (Martin and Ruberte, 1979).

The polyphenolic concentrations in the tubers were significantly correlated ($r = 0.89$) to the pattern of discoloration found in the yam tubers and their distribution within the tubers. The white yam had lower concentration on polyphenols (0.24–0.30 mg/g fresh tuber) than the water yam (0.48–0.58 mg/g fresh tuber) which showed the highest degree of discoloration. The fresh yam, however, had comparatively lower polyphenols ($P<0.05$) than the stored tubers although this may not be true for all the yam species. Thus, in the stored yams the highest degrees of discoloration and polyphenolic concentration were found in the distal ends of the tubers.

The correlation of discoloration value with the polyphenol content and their localization at the distal end of the yam tuber was similar to that observed in potato and other vegetables (Reeve et al., 1969; Matthew and Parpis, 1971; Karl, 1976; Mondy and Muller, 1977).

Differences in the amount and distribution of ascorbic acid, total lipid and total nitrogen were obtained for the fresh and stored tubers (Table 1). The fresh white yam had the highest amount of ascorbic acid (0.70 mg/100g) while the yellow yam had the lowest amount of ascorbic acid (0.54 mg/100g). In all the tubers, the stored yams generally had lower values for ascorbic acid than the fresh tubers. However total nitrogen levels in the stored tubers are generally lower than in the fresh tubers.

The tail region had the highest concentration of ascorbic acid while the head region had the least, with middle section having an intermediate value (not shown in table). Ascorbic acid decreased significantly ($P<0.05$) in the stored yam tubers both in gross amount and relative distribution within the tuber regions. The total lipid distribution among the tubers and within the tuber sections followed the same trend as ascorbic acid. The reduced levels in the total lipids and ascorbic acid of the tubers upon storage were similar to the trend observed for most vegetables.

The total nitrogen distribution in the fresh and stored tubers and sections is shown in Fig. 2. There was a significant decrease in the total nitrogen of the stored tubers compared to the fresh, but no trend was shown in the levels of nitrogen in the different sections of the tubers. The total nitrogen usually consists of non-protein and amino acids which may take part in the reactions leading to discoloration of yam products. In the cut raw fresh or stored yam tubers, susceptibility to discoloration may involve a number of chemical constituents and complex reactions as reported for potato (Monday et al., 1971) and the Chinese yam (Tono, 1970). Beyond varietal differences in the levels of the ascorbic acid, total acid, total lipids, and polyphenols, metabolic activity and loss of moisture continued in the stored tubers as in the fresh tubers. The variability in the storage characteristics of yam tubers may be responsible for differences in the amounts of metabolites which are involved in their susceptibility to the discoloration of the cut raw tuber or sensory properties of the products made from the tubers.

Evaluation of processed yam products

Color and texture constituted the major difference between the yam flour and the instant pounded yam flour. The mean scores for the yam flour and instant pounded yam flour reconstituted to the same consistency as the traditionally prepared foods are presented in Table 2. These products were made from fresh or stored tubers using whole peeled tubers of each species. The data reported are for the stored tubers because these yams are the most widely used for making products for preservation. Yam tubers, especially the head and tail regions, are not commonly used since the products made from them

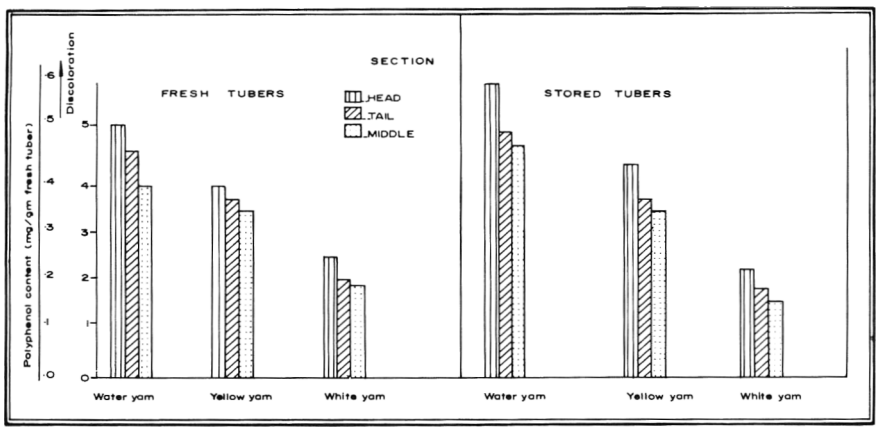

Fig. 1—Effect of polyphenol on the magnitude of discoloration in yam tubers.

Table 1—Proximate composition of yam tuber[a]

Determinations	Fresh yams			Stored yams		
	White	Yellow	Water	White	Yellow	Water
Moisture (%)	65	73	74	68	72	71
Dry matter (%)	28.9	27.6	24.8	25.5	26.5	23.5
Starch (%)	21.5	23.3	19.5	20.5	22.1	18.3
Crude fiber (%)	0.80	0.70	0.94	1.0	1.05	1.10
Total lipid (%)	1.10	1.38	0.92	0.90	1.00	0.70
Total nitrogen (%)	0.35	0.30	0.40	0.25	0.22	0.29
Ascorbic acid (mg/100g)	0.70	0.54	0.60	0.62	0.45	0.50
Ash (%)	1.45	1.34	1.25	1.45	1.34	1.25

[a] Data refer to average of two determinations on the peeled portions and are based on dry matter.

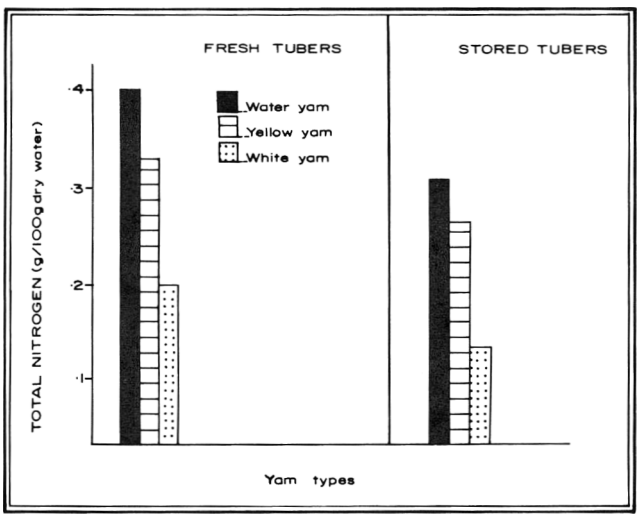

Fig. 2—Distribution of nitrogen in the fresh and stored yam tubers.

had variable color and texture and were rejected by the panelists.

The reconstituted instant pounded yam flour and yam flour products made from the fresh or stored white yam were rated highly in color, flavor and acceptability compared to similar products made from yellow yam or water yam. Mean scores for the products made from the fresh tubers shown in the first column (Table 2) were generally below five, indicating a slight dislike for these products made from fresh yams in terms of color and flavor compared to the same products made from the stored yams.

Significant differences in the color and flavor of the reconstituted products made from the tubers may be ascribed to the differences in the chemical constituents, especially polyphenol and total nitrogen, and in the manufacturing processes. The white yam had the least polyphenols and showed the least tendency towards discoloration unlike the water yam which had the highest level of polyphenols.

Discoloration in the cut yam tissue involves the action of phenoloxidases on the polyphenolic substances. For the instant pounded yam flour manufacture, the reaction leading to the discoloration was effectively controlled by the addition of sodium metabisulfite (250 ppm and the higher heat treatment 100°C for 10 min) to the diced yams, whereas the yams destined for yam flour (as is popularly called locally) were given a mild heat treatment of 60°C for 3 min.

The level of sodium metabisulfite 250 ppm and heat treatment combinations had previously been established to be the optimum conditions for obtaining the best colored instant pounded yam flour.

In typical yam flour production phenoloxidases were probably not inactivated, and the polyphenol concentrations and amino nitrogen reacted especially during the sun-drying stage leading to the formation of brown products. The laboratory-prepared yam flour simulated the local practice of manufacture in terms of heat treatment for enzyme inactivation and drying conditions. In a parallel study polyphenols in yam were completely inactivated above 90°C. Water yam with high polyphenols gave the best reconstituted yam flour as judged by the high rating for its color, flavor and acceptability. The dark brown color in reconstituted yam flour is an important index of acceptability and quality of the traditionally processed yam product especially among many people living in South-Western parts of Nigeria. The reconstituted instant pounded yam flour made with white yam was significantly preferred over other yams as judged by the highest scores for color and acceptability. White color is the most important attribute of reconstituted instant pounded yam flour or the traditionally prepared pounded yam. The highest scores for the color and

Table 2—Panel scores for the sensory attributes of the reconstituted yam flour and instant pounded yam flour[a,b]

	Quality attributes					
	Color		Flavor		Acceptability	
Tubers	Yam flour	Instant pounded yam flour	Yam flour	Instant pounded yam flour	Yam flour	Instant pounded yam flour
White yam	5.2a	8.1c	5.5e	6.2p	5.0z	7.2x
Yellow yam	5.1a	5.0d	4.3f	5.2p	5.0z	4.3k
Water yam	7.2b	4.2d	6.1e	4.1h	6.7y	4.3k

[a] Mean scores based on a 9-point hedonic scale where 9 = like extremely and 1 = dislike extremely. All the reconstituted products were made with the stored tubers.
[b] Values in a column not having a common letter are significantly different ($P<0.05$)

preference of the white yam product might be attributed to the low polyphenol content in this yam species compared to other types and the effectiveness of the method of controlling reactions leading to discoloration. The low scores of the products made from the yellow yam may be related to its bitterness and the slight yellow color of the tuber. The concentrations of polyphenols have been implicated in the astringent taste in potato (Mondy and Muller, 1977). The poor color and flavor of the yam products especially those made with the distal ends of the tubers may be attributed to their high polyphenol and nitrogen content. The reconstituted yam flour and instant pounded yam flour made from the middle section of stored white yam which had the least amount of polyphenols, total nitrogen and ascorbic acid were assessed by the panelists as having the best quality attributes of color and flavor. Although the yam flour is quite acceptable among the Yoruba speaking groups in West Africa, it is probably the only unique product where enzymatic discoloration of the tuber has been adopted to advantage. Most West African yam growers attempted to preserve their yams by the sun-drying technique to be milled later into flour. Since they had no adequate method for controlling the discoloration in the tubers, the products were accepted in their characteristic brown form, and most yams with a high tendency for discoloration are usually used for the manufacture of yam flour.

The yellow yam tuber is not normally used for the manufacture of instant pounded yam or yam flour but is cooked or roasted. The yellow yam is not as susceptible to enzymatic discoloration as the other varieties. Also, it is not suitable for processing into instant pounded yam because it contains bitter tasting components (Martin and Ruberte, 1975).

In products where uniform color and good flavor are the desired attributes yam tubers with low polyphenols should be used. This may explain the preference and significance attached to the white yam over yam cultivars (Coursey, 1967).

CONCLUSION

DIFFERENCES in the polyphenols content between fresh and stored yam tubers species and their distribution within the tuber were mainly responsible for their susceptibility to discoloration. The ascorbic acid, total lipid and total nitrogen distribution between and within the tubers may contribute to the discoloration of yam. Differences in polyphenols of the tubers were mainly responsible for the quality of the products made from the yam tubers. Commercial processors of yam products who wish to obtain and maintain a uniform quality of their product should select yams with low polyphenols and remove the distal portions (3–6 cm) of the tubers during peeling operation as these sections contain additional constituents that can affect the flavor of the products.

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Effects of Deaeration and Storage Temperature on Quality of Aseptically Packaged Guava Puree

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ABSTRACT

Deaeration of aseptically packaged guava puree did not benefit either color or flavor stability but did aid in the retention of ascorbic acid during the first 6 months of storage. The reduction of headspace O_2 was greatest in the samples stored at 23°C followed by lesser decreases in samples stored at 10° and −18°C. Sensory panel scores for color, Hunter color values, dissolved O_2, headspace O_2, and ascorbic acid were highly correlated with each other showing that color changes were related to O_2 consumption and ascorbic destruction.

INTRODUCTION

THE INTRODUCTION of bag-in-box aseptic packaging has provided new opportunities for marketing of products produced in areas distant from their major markets. Such is the case with many products produced in tropical regions which are destined for remanufacture elsewhere into final consumer products. Aseptic processing in bags offers cost savings in addition to flavor, color, and nutritive value improvement compared with canned products. Nelson (1984) reviewed the development of aseptic bag-in-box packaging of products for remanufacturing indicating that shelf-life remains a limitation for aseptically packaged products in flexible packages.

A problem encountered in storage of some aseptically packed products is browning, which limits shelf-life. Earlier work with bag-in-box aseptically packaged guava puree (Chan and Cavaletto, 1982) indicated browning was a problem that was initiated early in the product's storage at ambient temperature. This color defect was the limiting factor in product quality and was thought to be related to oxygen present in the puree or package.

Guava puree is rich in ascorbic acid with levels ranging from about 50 to more than 350 mg/100g depending on the cultivar. Degradation of ascorbic acid during storage may be responsible for browning of guava puree because ascorbic acid losses were found to accompany browning (Chan and Cavaletto, 1982). Numerous studies on orange juice and orange concentrate have shown the oxidative destruction of ascorbic acid and development of browning (Johnson and Toledo, 1975; Kanner et al., 1982; Kefford et al., 1959). During processing of guava puree a considerable amount of air is incorporated into the product. This air usually is not removed and may cause oxidation of ascorbic acid.

This study was designed to determine whether deaeration and cold storage would improve the stability of aseptically processed guava puree.

MATERIALS & METHODS

Processing and sample preparation

Guava puree (*Psidium gujava*, L.) (10,000 lb) was prepared at the Amfac Tropical Products plant, Keaau, HI, according to the method described by Boyle et al. (1957). Prior to aseptic processing, half of the puree was deaerated in a Dole centrifugal deaerator operating under vacuum (0.8 kg/cm^2) at 26°C. Both the deaerated and nondeaerated lots of guava puree (at 24–26°C) were heat processed in a Cherry-Burrell Thermutator at 93°C and held for 28 sec before cooling in a Thermutator at 27°C. The commercially sterile purees were then aseptically filled (Scholle, Model Auto-Fill X -1) into 1-gal polyester metalized bags (Scholle #804 AM), flushed with N_2 and capped. An initial quality evaluation was conducted and the remaining samples were stored at ambient temperature (22–24°C), refrigerated temperature (10°C), and frozen storage (-18°C) (deaerated puree only which was used as the control).

Samples were tested after 1, 3, 6, and 9 months. Five bags of puree were sampled for each treatment at each of the sampling periods. The frozen control was allowed to defrost overnight under ambient conditions. Prior to analysis each sealed bag of puree was massaged to mix the puree. Tests included dissolved and headspace oxygen, ascorbic acid, instrumental measures of color, pH, Brix, visual color evaluation of the puree, and taste tests on nectars made from the puree.

Oxygen determinations

The oxygen content in the headspace of the aseptically packaged puree was determined paramagnetically with a Servomex Oxygen Analyzer OA.570 (Sybron Taylor). Headspace oxygen was sampled by penetrating the aseptic bag cap. Dissolved oxygen was determined polarographically using a YSI 54ARC oxygen meter (Yellow Springs Instrument Co.) Dissolved oxygen readings were taken by placing the oxygen probe directly into the puree through the uncapped bags spout and moving the probe slowly in a circular motion through the puree.

Ascorbic acid assay

The high performance liquid chromatography method described by Watada (1982) was used for the determination of ascorbic acid. The ascorbic acid was extracted from guava puree with 6% metaphosphoric acid, chromatographically separated on a C_{18} cartridge in a radial compression module (Waters Assoc.) using 1.5% $NH_4H_2PO_4$ as a mobile phase in a high pressure liquid chromatograph (Tracor 995). The ascorbic acid concentration was based on the absorption of ultraviolet light at 245 nm with a Perkin-Elmer Model LC- 55 absorption detector.

Instrumental color readings

Color changes were measured by presenting a polystyrene petri dish (100 × 15 mm) containing 70g puree to the viewing port of the Hunter Colormeter D-25M-3 which was previously standardized with a pink tile (Hunter C2-8063). Results were expressed in Hunter *L, a, b* values.

pH and °Brix assay

pH was determined with a glass-electrode pH meter; °Brix was determined with a refractometer.

Sensory evaluation

A trained panel of 10 individuals evaluated the treatments for color, guava flavor intensity, and off-flavor after 0, 1, 3, 6, and 9 months of storage. All samples were evaluated on each of four days. Color was judged on single-strength puree and flavor on a drink that contained 20% puree which had been adjusted to 11°Brix with sucrose and 0.4% total titratable acidity with citric acid.

On the day of aseptic packaging the deaerated and nondeaerated purees were evaluated for color in comparison to the control (deaerated, nonfrozen puree). Thereafter, however, after each subsequent storage period, puree samples were compared with a control (deaer-

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ated) that was stored frozen, −18°C. In all tests, the subjects were asked to indicate if the treatment was more pink, a desirable attribute, (+1 to +3), the same as (0), or less pink (−1 to −3) than the control. Samples were placed in uncovered petri dishes and viewed under a Macbeth grading light.

Initially, drinks from the deaerated and nondeaerated purees were compared for flavor intensity and off-flavor. After each storage period, all samples, including an unidentified control, were rated on a 7-point flavor intensity scale where 1 = no guava flavor and 7 = intense, fresh guava flavor. Off-flavor was rated on a 7-point scale where 1 = intense off-flavor and 7 = no off-flavor. Drinks were prepared, chilled (5°C) and presented in random serving order in 50 mL beakers coded with random numbers. Tasting was done in individual taste booths equipped with white lighting.

Statistical analysis

All data were analyzed using programs in Statistical Analysis Systems (SAS) (Ray, 1982). Analysis of Variance (ANOVA) or the Generalized Linear Model (GLM) were used to determine significance between treatments. Means were compared where appropriate to determine significance by Duncan's Multiple Range Test. Simple correlation coefficients between chemical, physical, and sensory data were also calculated using the CORR program.

RESULTS & DISCUSSION

Effect of deaeration and storage temperature on color of aseptically processed guava puree during storage

During storage, Hunter "a" values decreased and "b" values increased indicating a change in hue to less red and more yellow (Fig. 1). This change became progressively more pronounced with each storage period at all storage temperatures. Storage temperature had the most significant ($p \leq 0.01$) effect on color changes with the highest storage temperature, 23°C, causing the greatest change in Hunter color values followed by less change at 10°C and even smaller changes in frozen samples at −18°C. Hunter "a" and "b" values for deaerated and nondeaerated samples were not significantly different during storage. Hunter "L" values which are values for the achromatic gray scale and therefore an indicator of darkening showed no significant difference ($p > 0.05$) between the deaerated and nondeaerated samples after aseptic processing and subsequent storage for 1 month at −18°C, 10°C, and 23°C (Fig. 2). However, there were significant ($p \leq 0.01$) changes in "L" values after 3 months storage between samples stored under cold storage temperatures (10°C and −18°C) and samples stored at room temperature (23°C). The puree stored at 23°C was darker after 3 months and continued to darken throughout the remainder of the study.

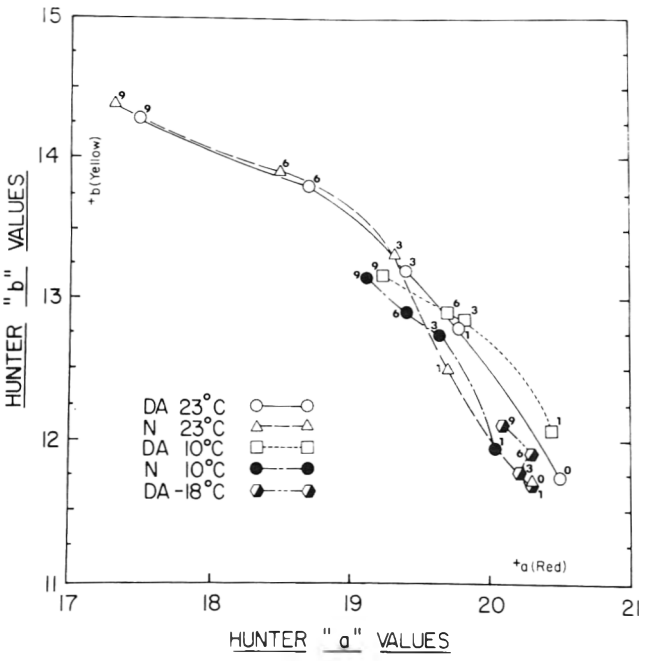

Fig. 1—Effect of deaeration and storage temperature on Hunter a *and* b *values in aseptically processing guava puree during storage: Superscripts designate 0, 1, 2, 3, 6, and 9 months storage, respectively; DA, deaerated; N, nondeaerated.*

Effect of deaeration and storage temperature on ascorbic acid retention in aseptically packaged quava puree

Analysis of the samples shows a slight but significant ($p \leq 0.01$) improvement in retention in ascorbic acid due to deaeration only in the 10°C sample after 6 months storage. Storage temperature had the greatest effect on ascorbic acid retention with the higher storage temperature 23°C having the most deleterious effect on ascorbic acid. This effect was evident after only 1 month. After 9 months storage at 23°C, the purees retained 42% of their ascorbic acid compared to retentions of 58–61% and 73% for purees stored at 10°C and −18°C, respectively (Fig. 3).

Effect of deaeration and storage temperature on oxygen content of packaged purees and their headspace

Dissolved oxygen content of deaerated and nondeaerated samples taken immediatedly after aseptic packaging were not significantly ($p > 0.05$) different statistically. Samples stored frozen at −18°C had significantly ($p \leq 0.05$) higher amounts of dissolved oxygen than those stored at 23°C. This is probably due to the slower rate of oxidative reactions occurring under frozen conditions thereby decreasing the amount of dissolved oxygen being reduced (Fig. 4).

The relative amount of oxygen existing in the headspace of the aseptic bag for deaerated and nondeaerated guava puree is shown in Fig. 5. For deaerated and nondeaerated samples the amounts of headspace oxygen in the aseptic bags sampled immediately after processing were not significantly different. However, the normal amount of oxygen in the atmosphere (21%) was reduced to 9.5% an 10.1% for the deaerated and nondeaerated samples, respectively. It is speculated that the bulk of the oxygen depletion occurred in both samples during heat processing where oxidation would accelerate due to the elevated temperature. Except for a slight increase in headspace oxygen in the frozen samples which may have been due to a

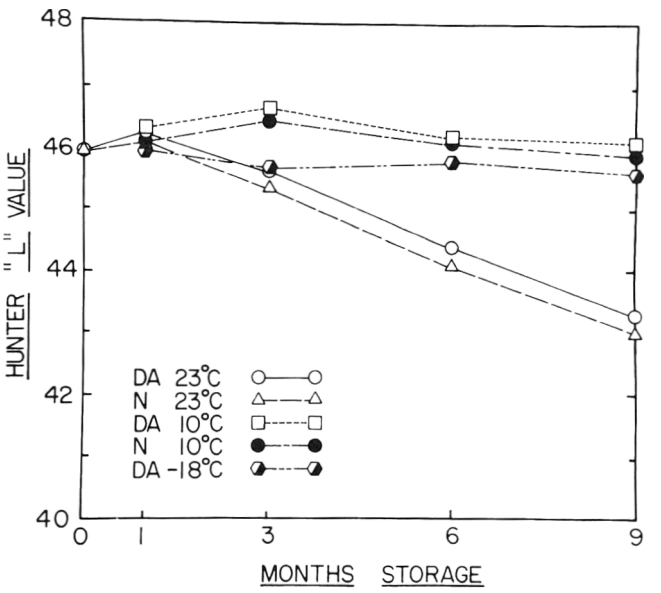

Fig. 2—Effect of deaeration and storage temperature on Hunter L *values in aseptically processed guava puree during storage: DA, deaerated; N, nondeaerated.*

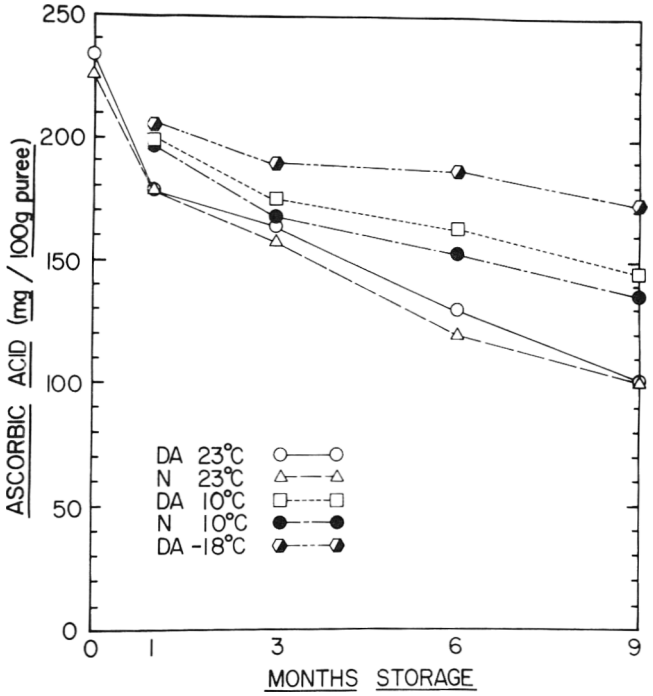

Fig. 3—Effect of deaeration, storage temperature and time on ascorbic acid retention in aseptically packaged guava puree: DA, deaerated; N, nondeaerated.

degassing effect caused by the freezing process the amount of headspace oxygen in all of the samples generally decreased during storage. The loss of headspace oxygen was most significant ($p \leq 0.01$) between samples stored under nonfrozen conditions (10°C and 23°C) and those in frozen storage (−18°C). The decrease in headspace oxygen can most likely be attributed to ongoing oxidative reactions occurring during storage. Except for the greater decrease in headspace oxygen after 1 month storage for the nondeaerated samples ($p \leq 0.01$) there were no significant ($p > 0.05$) differences for the remaining storage periods between deaerated and nondeaerated samples in rates of oxygen depletion. Storage temperature appeared to have the greatest effect on the rate of headspace oxygen depletion with the highest temperature 23°C resulting in the greatest amount of depletion followed by decreasing losses with decrease in storage temperatures at 10°C and −18°C.

Sensory evaluation

Color. No difference in sensory color scores was observed between deaerated and nondeaerated purees stored at the same temperature (Fig. 6). However, storage temperature had a significant effect on color, this effect becoming evident by 1 month. As the study progressed the puree stored at 10°C gradually became less pink than the frozen control, but the puree at 23°C was much less pink than either the control or the 10°C puree. By 3 months the difference in color between the 10°C and 23°C samples was highly significant ($p = < 0.01$) and this difference was observed for the remainder of the storage times. These findings are in close agreement with the objective color measurements (Fig. 1 and 2).

Flavor intensity and off-flavor. Flavor intensity was not seriously affected by the treatments or by storage time (Fig. 7). Some flavor intensity differences were observed in early storage but after 6 and 9 months, none was observed. Similarly, off-flavors which were thought would develop in parallel with deterioration in color did not develop to any great extent (Fig. 8). However, after 1 and 3 months, the control was judged to have slightly more off-flavor than the other treatments ($p \leq 0.05$ and ≤ 0.01, respectively). One possible explanation for this finding is that samples stored at 10°C and 23°C

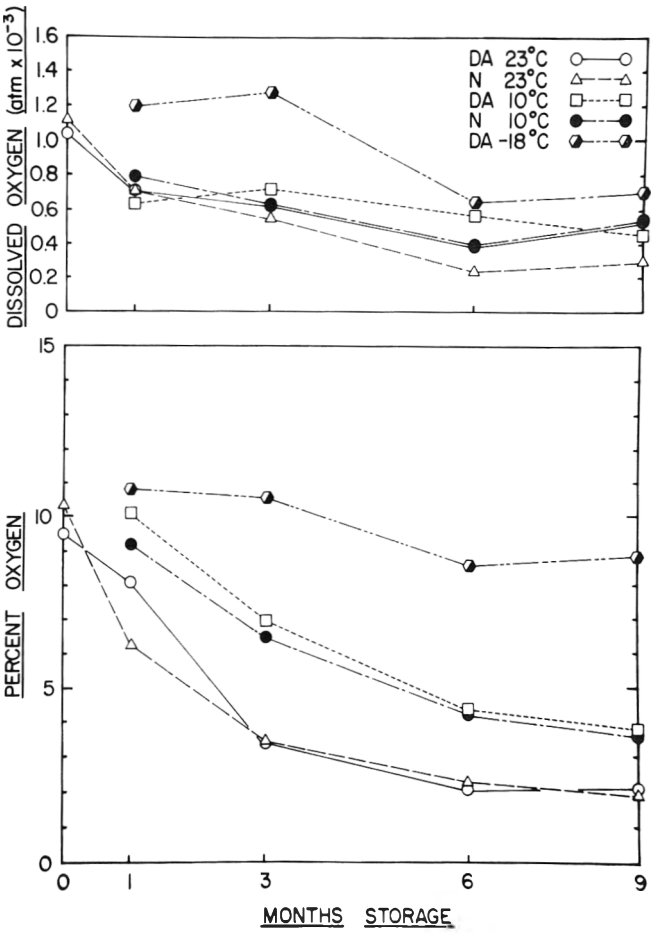

Fig. 4—Effects of deaeration, storage temperature and time on the dissolved oxygen content of aseptically packaged guava DA, deaerated; N, nondeaerated.

Fig. 5—Effect of deaeration, storage temperature and time on the headspace oxygen content of aseptically packaged guava puree: DA, deaerated; N, nondeaerated.

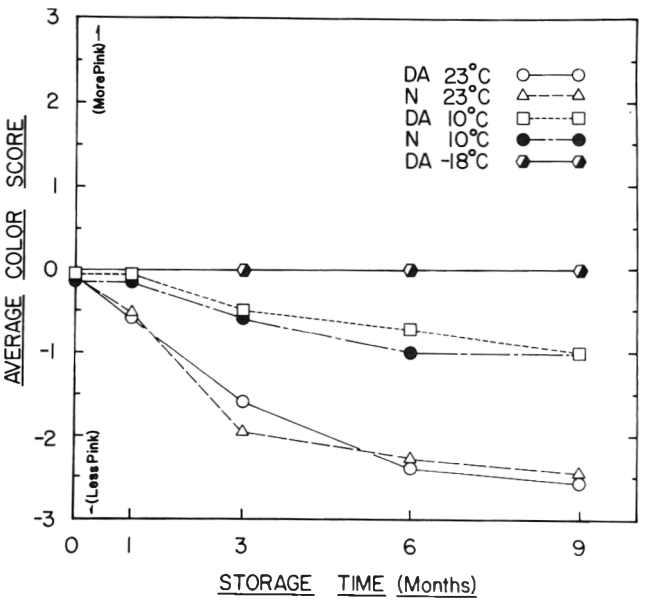

Fig. 6—Effect of deaeration, storage temperature and time on sensory color scores of aseptically packaged guava puree: DA, deaerated; N, nondeaerated.

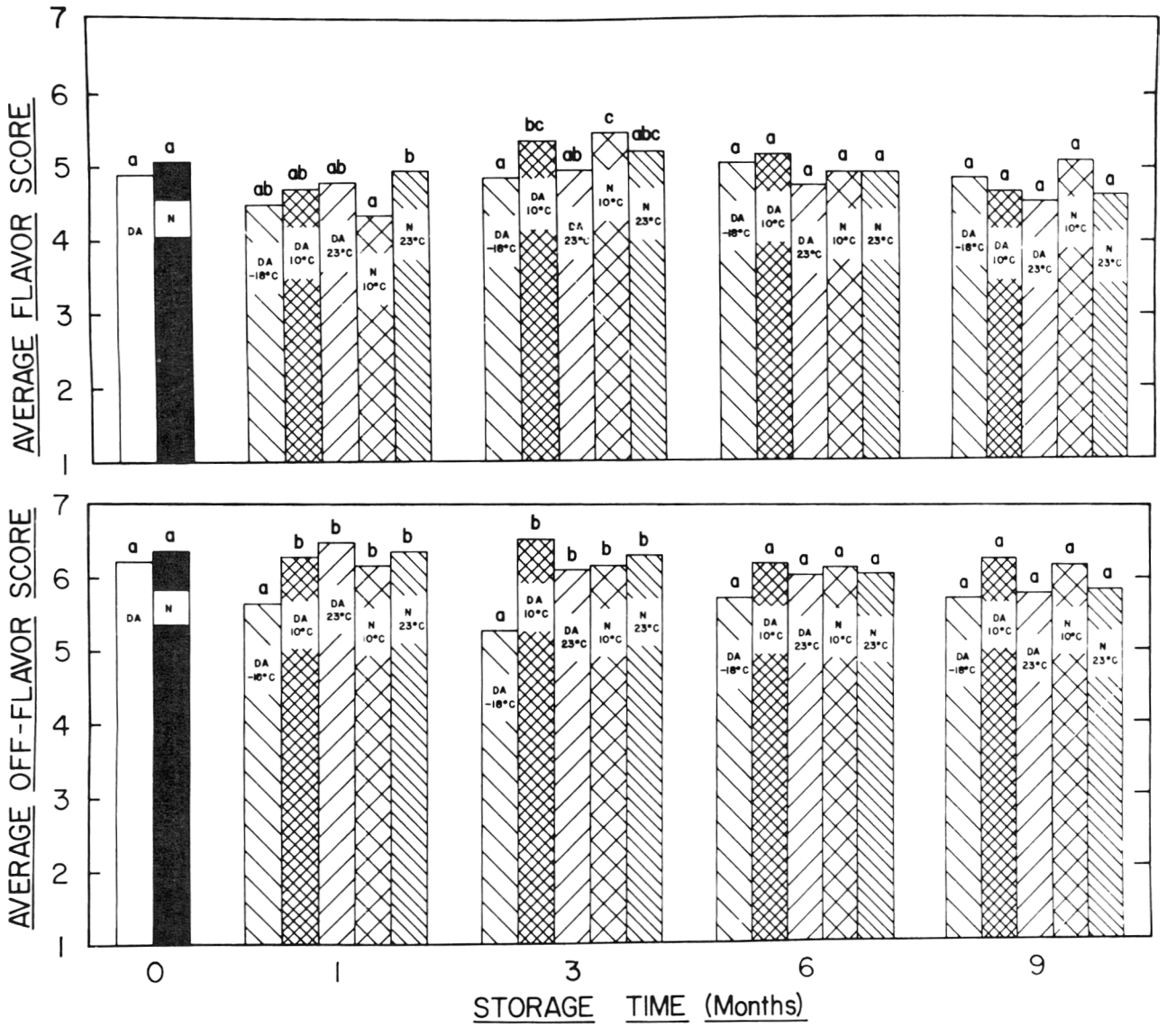

Fig. 7—Effect of deaeration, storage temperature and time on guava flavor intensity of drinks prepared from aseptically processed guava puree. Within any storage period treatments showing the same lower case letter are not significantly different from each other: DA, deaerated; N, nondeaerated. DA initial value, N initial value, DA −18°C, DA 10°C, DA 23°C, N 10°C, N 23°C.
Fig. 8—Effect of deaeration, storage temperature, and time on off-flavor development in drinks prepared from aseptically processed guava puree: DA, deaerated; N, nondeaerated. DA initial value, N initial value, DA −18°C, DA 10°C, DA 23°C, N 10°C, N 23°C. Within any storage period treatments showing the same lower case letter are not significantly different from each other

had developed "cooked" flavor that some panelist were accustomed to and may have considered more normal. After 6 and 9 months, no differences in off-flavors were observed between the treatments. These results should not be interpreted as "no flavor change," but rather that any changes that had occured were not in guava flavor intensity or in the development of off-flavor.

Correlation between chemical, physical, and sensory data

Sensory panel color scores were highly correlated with Hunter *L*, *a*, and *b* color values (r = 0.88, 0.92 and 0.95, respectively). A close association was also shown between sensory panel color and headspace oxygen (r = 0.89) and ascorbic acid (r = 0.89). Sensory panel color scores were also correlated to a slightly lesser degree with dissolved oxygen (r = 0.75) and with off-flavor (r = 0.72).

Hunter color "b" values also bore a close relationship with dissolved (r = −0.83) and headspace oxygen (r = −0.94) and ascorbic acid (r = −0.95) showing that color changes were related to the consumption of oxygen and the destruction of ascorbic acid.

Headspace oxygen and dissolved oxygen were closely related (r = 0.86) and these both were correlated with ascorbic acid retention (r = 0.88 and 0.81, respectively).

Correlation coefficients between sensory panel flavor scores and other measures were all low. The same was true for off-flavor scores with the exception of the correlation with sensory panel color previously mentioned. The correlation between flavor intensity scores and off-flavor scores was also low (r = 0.34).

CONCLUSIONS

THE QUALITY of aseptically processed bag-in-box guava puree can best be assured by lowering product temperature during storage. Storage at 10°C will retard, but not prevent, color change and loss of ascorbic acid. Cold storage will increase the puree cost, but would be desirable if lengthy storage is anticipated. Deaeration of the puree had almost no effect on the initial dissolved oxygen levels in this study, hence little or no differences in quality were observed between deaerated and nondeaerated purees during storage. However, Kefford et al. (1959) and Passy and Mannheim (1979), also concluded that

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Desorption Isotherms for Plantain at Several Temperatures

O. O. AJIBOLA

ABSTRACT

Moisture equilibrum data for desorption of water from ripe and unripe plantain was obtained at 40°, 50°, 60°, and 70°C over a range of relative humidities from 10–90% using a static gravimetric method. Ripe plantain exhibited higher equilibrum moisture (EMC) than unripe plantain when exposed to the same conditions. A nonlinear least squares regression program was used to fit five desorption isotherm models to the experimental data and the parameters of the models evaluated. The minmum standard error of estimates of the EMC (1.9% for ripe and 1.4% for unripe plaintain) was obtained by using the modified Halsey model.

INTRODUCTION

PLANTAIN (*Musa paradiasca*) is an important source of carbohydrate in the humid tropics. It is a seasonal crop being plentiful at some times of the year and scarce at other times. The fruits can be allowed to ripen at ambient temperature before being processed for consumption or dried and milled into flour in the unripe stage. Because of lack of cheap practical storage techniques for plantain, most of the fruit ripen at about the same time leading to high post-harvest losses which could be from 35–100% (NAS, 1978). Several methods of conserving plantain are presently being considered and investigated. These methods include drying, transformation into chips, powder and flour (Ogazi and Jones, 1981). These processes invariably involve the drying and storage of the crop. A fundamental characteristic of food materials which influences every aspect of the drying process and the storage stability of the dried product is its water sorption characteristics. For this reason, there have been many scholarly contributions which deal with the experimental means to obtain sorption isotherms. In one such contribution, Gal (1981) pointed out that there are three basic methods of determining sorption data; namely, manometric, gravimetric, and special methods. He concluded that the gravimetric method was preferred for obtaining complete sorption isotherms. These data are available for a wide variety of products (ASAE, 1981) but are not available for plantain.

Many other researchers have developed and tested models of the form:

$$MC = MC\ (RH,T)$$

and

$$RH = RH\ (MC,T)$$

where T = temperature, MC = moisture content at equilibrum, and RH = relative humidity at equilibrum. A recent review of the models was done by Bruin and Luyben (1980).

Thc objectivc of this study was to obtain data on desorption isotherms of ripe and unripe plantain at 40°, 50°, 60°, and 70°C and to interpret the data in terms of established sorption models. This is useful in many aspects of drying where the user is interested in the model which fits the experimental data as closely as possible.

MATERIALS & METHODS

THE PLAINTAIN used for this study was obtained from the University of Ife Research Farm. Ripe and unripe fruits were investigated. The samples were peeled, cut into pieces about 1.5 cm thick and dried in an oven until the weight was reduced by about one-third. This drying technique was found necessary to reduce the length of time required to reach equilibrum and also lessened the amount of excess salt needed in the saturated salt solutions. A static gravimetric technique was used for the equilibrum moisture determination. Samples of about 20g were placed in wire baskets which were then placed in desiccators. Each desiccator contained a saturated salt solution, the set of solutions being selected to provide adequate screening of air relative humidities from 10 to 90% at the different temperatures (Young, 1967). The desiccators were placed in ovens at 40°, 50°, 60°, and 70°C. The oven temperatures were monitored and controlled to within ± 1.0°C. The samples were weighed daily and equilibrum conditions were considered reached when three consecutive measurements gave identical readings. The dry matter of the samples was obtained by drying in an oven at about 90°C to constant weight. At this temperature the samples dried without caking. Each experiment was replicated.

RESULTS & DISCUSSION

THE EQUILIBRUM moisture data, expressed as percent on dry basis, at different temperatures and relative humidities are presented in Fig. 1 for ripe plantain and Fig. 2 for unripe plantain. The data indicated that the equilibrum moisture of ripe plantain was higher than that of unripe plantain at all temperatures and for all relative humidity values tested. It has been shown that during ripening of plantain, complex carbohydrates, especially starch, are hydrolyzed to reducing sugars and sucrose and that there is an increase in the moisture of the fruit (Ketiku, 1973). Crapiste and Rotstein (1982) in a study of the sorptional equilibrum data of starch containing foodstuff concluded that a product with a large amount of solute and less insoluble materials has less equilibrum water at low relative humidities but rather more at high relative humidites. This was demonstrated in comparisons of equilibrum moisture data for beans with potatoes and peas with corn. Mazza (1982)

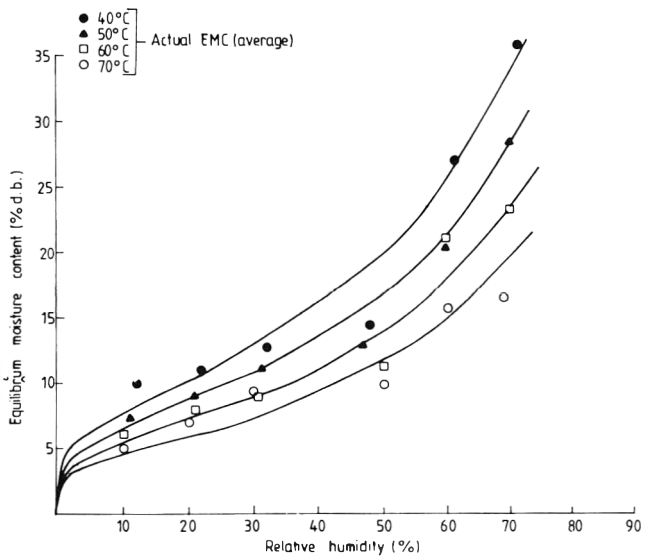

Fig. 1—Actual average equilibrium moisture content data for ripe plantain and predicted isotherms using modified Halsey model.

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Table 1—Equilibrum moisture content and equilibrum relative humidity models

Name of model	Equilibrum moisture content models	Equilibrum relative humidity model
Henderson (Henderson, 1952)	$M = \left[\text{Ln}\frac{(1-RH)}{-aT}\right]^{(1/b)}$	$1 - RH = EXP(-aTM^b)$
Chung-Pfost (Chung and Pfost, 1967)	$M = \frac{-1}{b}\text{Ln}\left[\frac{T\,\text{Ln}\,RH}{-a}\right]$	$RH = EXP\left[\frac{-a}{T}EXP(-bM)\right]$
Modified Halsey (Iglesias and Chirife, 1976)	$M = \left[\frac{EXP(a-bT)}{-\text{Ln}\,RH}\right]^{1/c}$	$RH = EXP[-EXP(a+bT)M^{-c}]$
Chen-Clayton (Chen and Clayton, 1971)	$M = \frac{-1}{cT^d}\text{Ln}\left[\frac{\text{Ln}\,RH}{-aT^b}\right]$	$RH = EXP[-aT^b EXP(cT^dM)]$
Henderson-Thompson (Thompson, 1972)	$M = \left[\frac{\text{Ln}(1-RH)}{a(t+b)}\right]^{1/c}$	$1 - RH = EXP[-a(t+b)M^c]$

a, b, c, d = perameters of the models
M = moisture content, % dry basis
RH = relative humidity, decimal fraction
T = temperature, k
t = temperature, °C

has also shown that the addition of glucose and sucrose to potato caused the equilibrum moisture to decrease in the low and intermediate relative humidities range while increasing significantly at the high relative humidities range. The higher equilibrum moisture values of ripe plantain at low and high relative humidities were unexpected. This however could be due to the effect of other changes that occur during ripening. These changes include decreases in acidity, starch and ascorbic acid, and increases in pH, ash and fiber (Ketiku, 1973).

A nonlinear, least squares regression program GENSTAT OPTIMIZATION (NAG, 1981) was used to fit five desorption models (Table 1) to the experimental data. Parameters were estimated for the model first by taking the equilibrum relative humidity (ERH) as the dependent variable and secondly by taking the equilibrum moisture content (EMC) as the dependent variable.

The estimated parameters for the ERH model with the standard errors of estimate for relatively humidity are shown in Tables 2 and 3 for ripe and unripe plantain, respectively. The estimated paramaters for the EMC models with the standard errors of estimate of moisture are shown on Tables 4 and 5 for ripe and unripe plantain, respectively. Comparisons of the parameter values in Tables 2 and 4 and Tables 3 and 5 show that the parameters obtained with the EMC models are not the same as the corresponding parameters in the ERH models.

For ripe plantain the lowest standard error of estimate for ERH (5.9%) and EMC (1.9%) was obtained by using the modified Halsey model (Tables 2 and 4). For the unripe plantain the lowest standard error of esimate of 6.4% for ERH was obtained by using the modified Henderson model while the lowest standard error of estimate of EMC (1.4%) was obtained using the modified Halsey model. Fig. 1 presents the desorp-

Table 2—Comparison of equilibrum relative humidity models for ripe plantain

Model	Parameters a	b	c	d	Standard error of estimate (%)
Henderson	7.075×10^{-5}	1.210			9.6
Chung-Pfost	8.84×10^{2}	8.10×10^{-2}			10.1
Modified Halsey	9.83	-2.019×10^{-2}	1.321		5.9
Chen-Clayton	4.797×10^{11}	−4.457	5.037×10^{-4}	8.900×10^{-1}	8.7
Modified Henderson	-3.5×10^{-4}	1.361	9.410		7.5

Table 3—Comparison of equilibrum relative humidity models for unripe plantain

Model	Parameters a	b	c	d	Standard error of estimate (%)
Henderson	7.553×10^{-5}	1.450×10^{-1}			8.5
Chung-Pfost	1.098×10^{3}	1.574×10^{-1}			8.3
Modified Halsey	1.030×10^{1}	-2.161×10^{-2}	1.612		8.0
Chen-Clayton	5.371×10^{6}	−2.453	9.378×10^{-5}	1.286	7.3
Modified Henderson	-3.9×10^{-4}	1.562	−4.61		6.4

Table 4—Comparison of equilibrum moisture models for ripe plantain

Model	Parameters a	b	c	d	Standard error of estimate (%)
Henderson	5.2×10^{-5}	1.319			3.9
Chung-Pfost	1.123×10^{3}	9.407×10^{-2}			4.1
Modified Halsey	1.049×10^{1}	2.272×10^{-2}	1.252		1.9
Chen-Clayton	9.953×10^{2}	−2.173	1.593×10^{-5}	1.496	3.4
Modified Henderson	3.6×10^{-4}	1.370	-1.172×10^{1}		2.6

Table 5—Comparison of equilibrum moisture models for unripe plantain

Model	Parameter a	b	c	d	Standard error of estimate (%)
Henderson	1.157×10^{-4}	1.278			2.1
Chung-Pfost	1.042×10^{3}	1.446×10^{-1}			2.2
Modified Halsey	1.012×10^{1}	1.999×10^{-2}	1.778		1.4
Chen-Clayton	1.71×10^{7}	−2.67	2.761×10^{-5}	1.481	1.8
Modified Henderson	5.2×10^{-4}	1.359	6.275		1.6

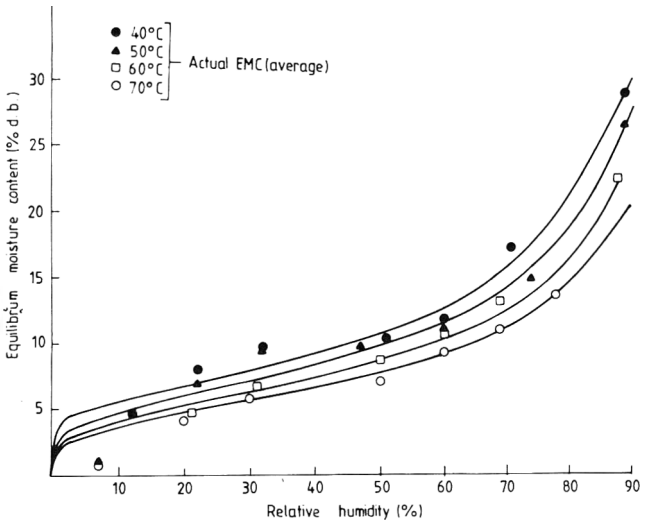

Fig. 2—Actual average equilibrium moisture content data for unripe plantain and predicted isotherms using modified Halsey model.

tion isotherms using the modified Halsey model and the average of the actual data for each relative humidity and temperature studied for ripe plantain. Fig. 2 presents the same information for unripe plaintain.

CONCLUSIONS

THE EMC of ripe plaintain was higher than that of unripe plaintain at all conditions. The minimum standard error of estimate of EMC obtained by using the modified Halsey model was 1.9% for ripe plaintain and 1.4% for unripe. The minimum standard error of estimate of ERH obtained with the modified Halsey model was 5.9% for ripe plantain; the minimum standard error of estimate of ERH when determined by modified Henderson model was 6.4% for unripe plantain. This information may be useful to processors involved in the drying of plantain.

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ASEPTICALLY PACKAGED GUAVA PUREE QUALITY. . .From page 168

deaeration of citrus juices had little effect on retention of product quality during storage and the governing factor of quality deterioration with time is storage temperature. Evidence suggests that available oxygen in the puree and package is consumed during storage, resulting in destruction of ascorbic acid and in product browning.

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Heat Induced Browning of Clarified Apple Juice at High Temperatures

J.L. TORIBIO and J.E. LOZANO

ABSTRACT

A procedure to determine deterioration reaction kinetics of fluid foods at elevated temperatures is described. With this method the nonenzymatic browning (NEB) measured as O.D. at 420 nm of Red Delicious (RD) and Granny Smith (GS) apple juices was examined. Color development at 15°, 30°, 50°, and 70° Brix and temperatures ranging from 90–108°C were compared over 60 min. Results indicated an apparent first order reaction rate depending on temperature, apple juice composition and soluble solids. Browning occurred at a more rapid rate in GS juice. NEB reaction rate was shown to depend critically on the total amino acid content. Temperature dependence followed the Arrenhius equation and the activation energy (E_a) ranged from 22.0–24.8 Kcal/mole.

INTRODUCTION

THE OBJECTIVES of apple juice concentration are mainly to reduce costs and to increase shelf life. This operation has, however, side-effects which adversely affect the juice quality. Losses of aroma, changes in color and development of undesirable flavors are common quality degrading side-reactions and nonenzymatic browning (NEB) is the most significant parameter for commercial acceptability.

Concentration by evaporation ideally removes water without changes in solids composition; however, in practice clarified apple juice darkens during evaporation. Brown color development results from reactions between reducing sugars and amino acids forming brown pigments (Pribella and Betusowa, 1978; Toribio and Lozano, 1984).

The study of the problem may be simplified by assuming that all the reactions which produce an increase in juice darkening are related to temperature, soluble solids and heating time. The concentration process may be optimized if the effect of temperature and moisture upon these reactions and properties is known. Evaporation can be done under an extremely wide range of temperature and time combinations. Multiple effect evaporators used in apple juice processing plants were designed to eliminate water under vacuum at reduced temperatures. This would be the ideal situation but in actual practice temperatures become very high at the first effect (Lozano et al., 1984). Little is known about the actual residence time distribution of the juice as it undergoes nonisothermal concentration.

Previous literature on quality determination of fruit juices was addressed to storage or low temperature processes. The available literature on quality changes during concentration is mainly devoted to citrus juices (Saguy et al., 1978a, 1978b). Recently, Beveridge and Harrison (1984) determined brown color development in a pear juice concentrate previously decolorized with activated charcoal. The maximum temperature at which the pear juice was heated was 80°C.

The objectives of this investigation were: (1) to develop an experimental procedure that could be used to obtain kinetic information on heat-induced color degradation of juice and (2) to present data on nonenzymatic browning of clarified apple juice, as a function of heating time, temperature and soluble solids. The temperatures screened corresponded to the highest temperatures found in commercial apple juice evaporation.

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MATERIAL & METHOD

THE JUICES studied were manufactured from the two prevailing apple varieties processed in Argentina, Red Delicious (RD) and Granny Smith (GS). Single strength clarified apple juices were made following the experimental procedure described in previous work (Toribio and Lozano, 1984). Concentration was performed in a Labconco freeze dryer to minimize deterioration. The condenser was kept at −80°C and the sample flask at room temperature. The juices were concentrated to 75° Brix. Various degrees of soluble solids samples were made by reconstituting the 75° Brix concentrate.

Physical analyses

Soluble solids were measured at °Brix with a bench refractometer at 20 ± 0.1°C. Color was determined as O.D. at 420 nm in samples pre-diluted to 12.5° Brix with distilled water. Absorbance was determined against water on a Perkin-Elmer Lambda 3 spectrophotometer using 1 cm cells. No significant change in turbidity (as O.D. at 625 nm) after heat treatment was observed.

Chemical analyses

Total amino acids (AA) were determined using the formol titration method (AOAC, 1980). Total acidity (TA), pH, total sugars (TS) and reducing sugars (RS) were also estimated according to AOAC methods; total phenolics (TP) were determined using the Folin-Ciocalteau reagent (Singleton and Rossi, 1965).

Heating and cooling procedures

The experimental apparatus consisted of a set of thin rectangular cells, made of stainless steel. The cells had 5 mL capacity with an exposed area of approximately 104 cm^2. Two 10 mL Luer-lock type syringe needles were soldered to each cell top for feeding and removing the samples.

The temperature of the juice was measured with a 0.42 mm Teflon isolated type K thermocouple connected to a Fluke 2190 Digital Thermometer and a Varian 9176 Recorder. Fast cooling after the treatment, necessary to drastically reduce the nonenzymatic browning reactions, was obtained by sinking the cells in a −10°C ETOH/water bath (Cole Parmer K-1268). Up to ten cells, previously filled with apple juice, were placed in an appropriate aluminium rack which was located in the heating bath (Colora Ultrathermostat) such that the cells penetrated 9 cm into the heating medium. The medium was a glycerol/water solution making it feasible to reach temperatures up to 110°C and facilitating heat transfer to the cell. Each cell was transferred to the cooling medium at selected times. Transfer time was about 2 sec.

RESULTS & DISCUSSION

THE TECHNIQUE of heating small samples of foods in glass and aluminum tubes or plastic bags to simulate the effect of heat on quality has been widely used (Farkas, 1962; Odlaug and Pflug, 1977; Erdtsieck and Beumer, 1976). Saguy et al. (1978a) used a more sophisticated steam-heated thermal unit. In the present study the experimental setup was designed to meet the following basic requirements: (1) short come-up time; (2) constant temperature and soluble solids during heating; (3) relatively high sample capacity. None of the above methods could satisfy all these requirements simultaneously.

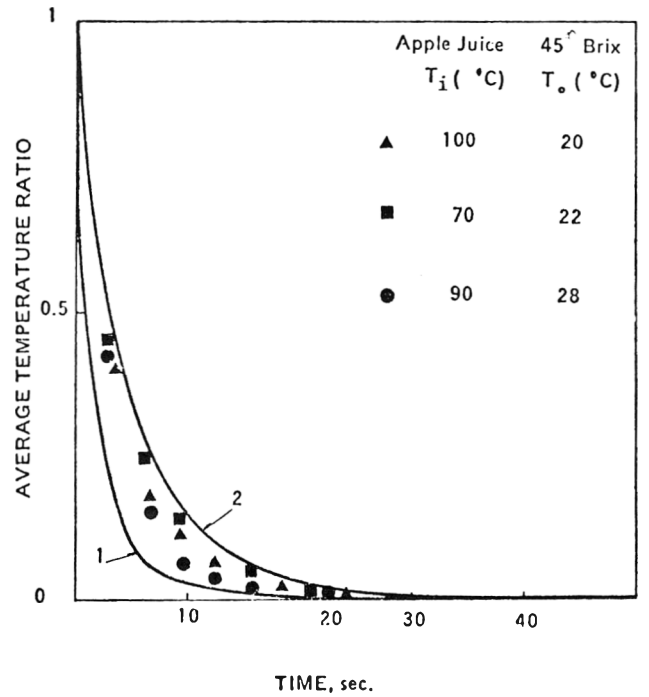


Fig. 1—Unsteady state heating in the cells. Average temperature ratio vs time; T_o and T_i are defined in Eq. (1); curve 1 represents Eq. (1) for a 15° Brix juice; curve 2 represents Eq. (1) for a 70° Brix juice.

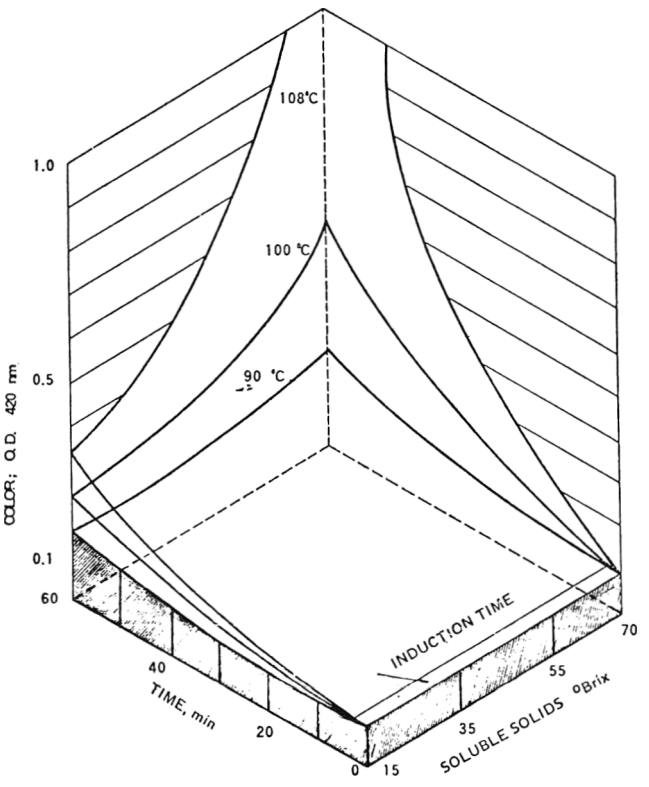


Fig. 2—Color as a function of heating time, temperature and soluble solids (Granny Smith).

The time required to reach a given temperature at a certain point of a conductive body can be calculated, provided the geometrical shape as well as the initial and boundary conditions are known. For calculations purposes the cell and its contents are regarded as an infinite slab bounded by two parallel planes. This is a classical problem in heat transfer and has been solved (Carslaw and Jaeger, 1959; Bennett and Myers, 1962; Crank, 1975). Under the present conditions the average temperature of the juice filling the cell can be calculated from (Loncin and Merson, 1979):

$$\theta = \frac{T_i - \bar{T}}{T_i - T_o} = \frac{8}{\pi^2} \sum_{n=1}^{\infty} \frac{1}{(2n-1)^2} \exp - \left[(n - 1/2)^2 \pi^2 \frac{(\lambda/\rho c_p)}{\Delta x^2} t \right] \quad (1)$$

where T_i = heating medium temperature; $\bar{T}$ = average juice temperature at time t; T_o = initial juice temperature; and θ = average temperature ratio.

The density (ρ), heat capacity (c_p) and thermal conductivity (λ) of the juice were considered to be equal to those of a sucrose solution of the same concentration as the juice (Honig, 1953). They were considered constant over the entire range of temperature. Fig. 1 shows measured temperature increase of an apple juice at 45° Brix with heating time at various heating medium temperatures expressed as average temperature ratio, θ, defined in Eq. (1). Influence of soluble solids as predicted by Eq. (1) is also shown in Fig. 1. No more than 40 sec were

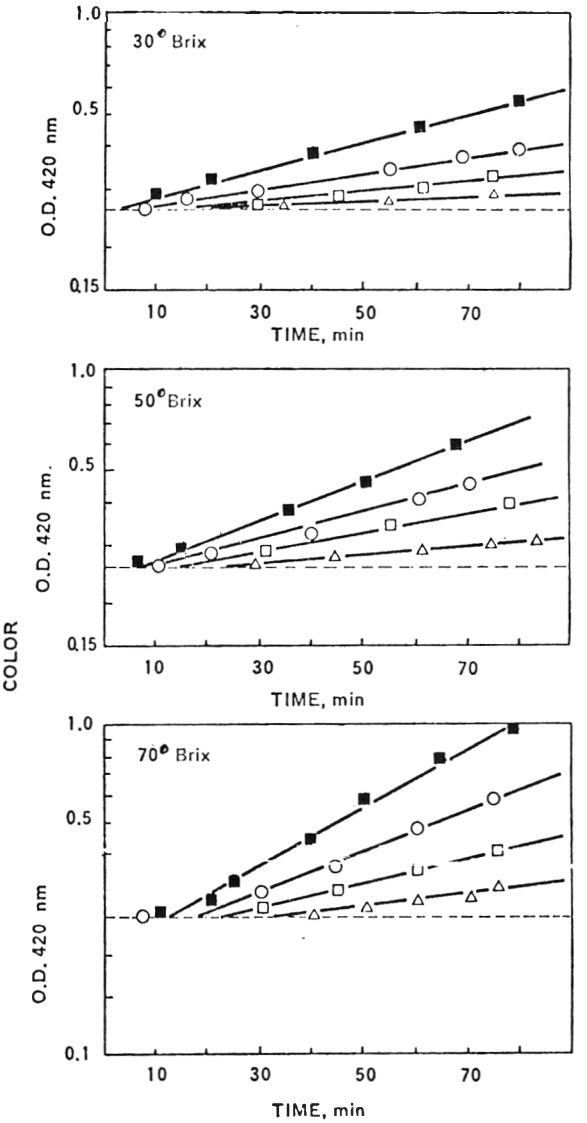


Fig. 3—Color as a function of heating time (Red Delicious) ■ 108°C; ○ 104°C; □ 100°C, and △ 90°C.

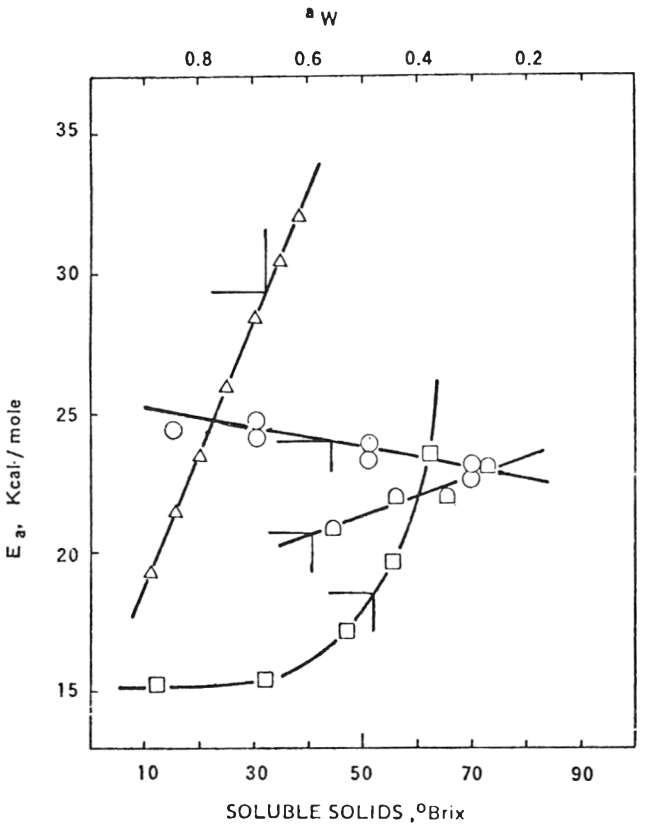
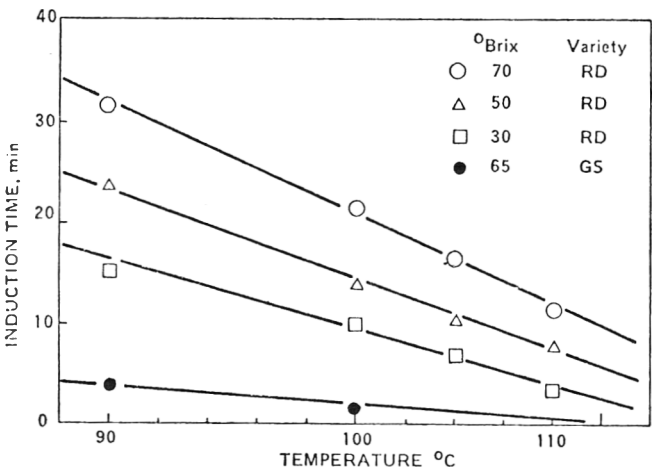


Fig. 4—Moisture dependence of the activation energy for non-enzymatic browning. △ Intermediate moisture foods (Singh et al., 1983); ▵ Pear juice (Beveridge and Harrison, 1984); □ Grapefruit juice (Saguy et al., 1978a); ○-This study.



Effect of temperature and soluble solids on the induction (τ) time period for Red Delicious and Granny Smith apple juices.

necessary to reach θ = 0.005, which represented a very good approach to the final average juice temperature.

Color increase of Granny Smith apple juice during heating at various concentrations and temperatures is shown in Fig. 2. Similar results obtained from experiments with Red Delicious apple juice are plotted in a semilog graph (Fig. 3) to better appreciate the time needed for the coloring reactions to become detectable. These results indicated that browning increased exponentially with time after an initial induction period which depended on soluble solids, the temperature and the juice variety. This relationship represented first order kinetics with a

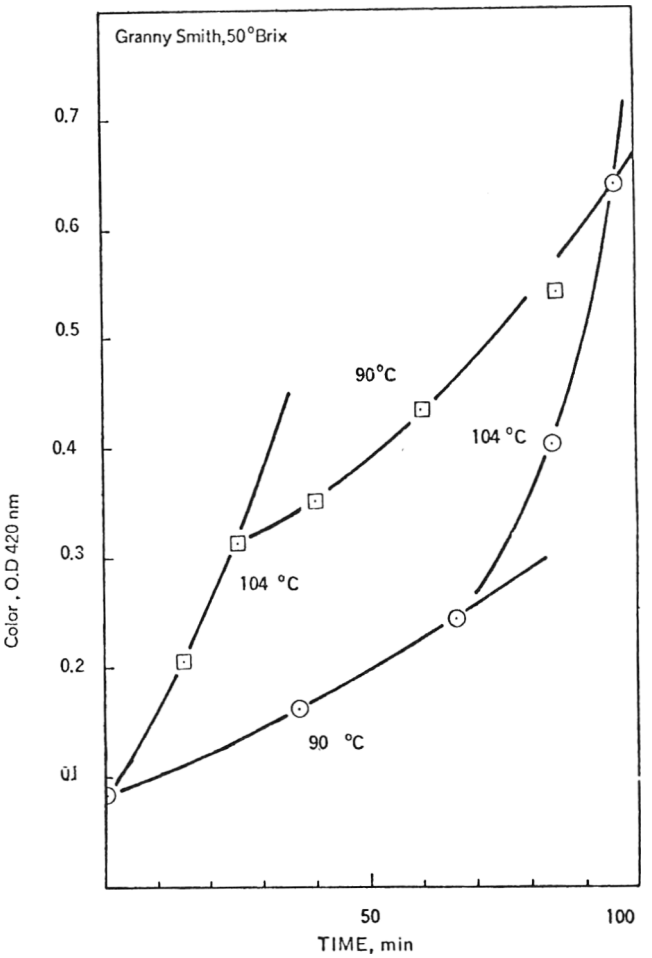


Fig. 6—Effect on the browning rate of the initial color; Full lines represent Eq. (2).

Table 1—Physical and chemical analyses

	Granny Smith	Red Delicious
pH	3.51	4.02
Total acidity (g/L)	6.0	2.2
Reducing sugars (g/kg)	89.0	94.5
Total sugars (g/kg)	104.4	112.6
Total phenolics (ppm)	6.59	7.35
Total amino acids (meq/L)	7.8	2.6
Initial color (O.D.420 nm)	0.095	0.255

lag-period. Initial lag period in browning of other fruit juices and model systems has been reported (Karel and Nickerson, 1964; Clegg and Morton, 1965; Legault et al., 1951; Saguy et al., 1978a,b; Reyes et al., 1982) It was explained as the time during which colorless intermediates were formed. For these kinetics, the color development can be expressed as:

$$C = C_o \exp[-K(t - \tau)] \qquad t > \tau \qquad (2)$$

where K = reaction constant; C_o = initial color; τ = induction time; and t = time of heating.

Physical and chemical characteristics of juices, including initial color, are listed in Table 1. Calculated lag times and K values with the associated coefficients of determination (r^2) are summarized in Tables 2 and 3.

Lag times were only significant for RD samples. Fig. 4 shows the effect of temperature and soluble solids on τ with variety as a parameter. As water content increased the lag period became shorter. Labuza et al. (1970) suggested this effect on τ could indicate that formation of browning pigments had a different pathway at lower water content.

Tables and 2 and 3 also show that Red Delicious and Granny

Table 2—Effect of temperature and soluble solids on the rate of browning formation of Granny Smith juice

Soluble solids (°Brix)	Temp (°C)	Induction time (min)	Rate constant $(min^{-1}) \times 10^3$	Detm. coef. r^2	Activation energy (Kcal/mole)
15	94	—	6.345	0.986	24.8
	100	—	11.39	0.988	
	104	—	17.13	0.982	
	108	—	21.52	0.992	
30	90	—	6.33	0.991	24.3
	94	—	9.47	0.979	
	100	—	15.56	0.967	
	104	—	21.92	0.979	
	108	—	30.97	0.996	
50	90	—	8.98	0.960	23.4
	100	—	22.45	0.978	
	104	—	31.42	0.990	
	108	—	40.91	0.992	
69	90	4	12.39	0.982	22.4
	100	1,3	27.90	0.988	
	101	—	31.55	0.992	
	104	—	41.60	0.988	
	108		52.20	0.994	

Table 3—Effect of temperature and soluble solids on the rate of browning of Red Delicious

Soluble solids (°Brix)	Temp (°C)	Induction time (min)	Rate constant $(min^{-1}) \times 10^3$	Detm. coef. r^2	Activation energy (Kcal/mole)
30	90	15.0	1.87	0.974	24.7
	100	10.0	4.60	0.982	
	104	7.0	6.71	0.942	
	108	3.5	9.45	0.988	
50	90	23.5	3.25	0.976	12.2
	100	13.5	6.73	0.969	
	104	10.0	10.80	0.959	
	108	7.9	14.90	0.981	
70	90	31.6	4.70	0.986	22.0
	100	21.5	9.95	0.964	
	104	16.5	14.37	0.988	
	108	11.5	20.00	0.974	

Smith apple juices brown at different rates under the same conditions, the lightest juice (GS) coloring faster than the other (RD). This behavior could be explained by considering the corresponding juice composition. It is well known that NEB reactions are greatly influenced by water content, pH, temperature, type of sugars and amino-acids. The effect of pH was not drastic over the narrow range of values reported in this work (Table 1). The same is true for reducing sugars and polyphenols. On the other hand, differences in AA content were significant. When Maillard reactions occur, the amino acids are limiting substances and greatly influence reaction rates. It can be calculated from Tables 1 and 2 that for a three times greater AA content, there is an approximate threefold increase of the reaction constant K. A similar pattern was also found during storage at different temperatures of apple juice concentrate (Toribio and Lozano, 1984). This suggests that monitoring of AA can be used as a quick method for estimating potential browning during processing and storage whatever the apple juice variety.

The Arrenhius activation energy (E_a) values are also given in Tables 2 and 3. The effect of apple variety was not significant. It has been suggested that activation energy depends on soluble solids content (Labuza and Saltmarch, 1981; Singh et al., 1983; Beveridge and Harrison, 1984). Correlating the experimental data gives:

$$E_a = 25.874 - 0.0516\ X \qquad (3)$$

$$r^2 = 0.977$$

The calculated precision of the E_a result is 6% (Hill and Grieger-Block, 1980). Therefore, the moisture dependence is open to question since the values spanned by Eq. (3) in the experiments described differ about 12%.

Comparing these results and those previously reported by other authors for whey powder, intermediate moisture apples and pear juice concentrate (Fig. 5), the E_a dependence on X is quite different in the different systems. Reasons for such behavior are not clear. The data seemed to indicate that a level of about 22 Kcal/mole prevailed more or less independent of the soluble solids. The scatter of E_a may be due to diffusion limitation effects in some of the systems. Brüin and Luyben (1980) noted that the generally accepted pseudo-zero order NEB reaction should be used with care, because the above mentioned diffusion limitations may mask the concentration dependence of the reaction rate.

A further study was made to evaluate the effect of initial color on the browning rate. Fig. 6 shows the brown color development of a GS sample after 25 min of thermal treatment of 104°C followed by 58 min at 90°C and, inversely, another sample of the same variety heated for 66 min at 90°C and then for 30 min at 104°C. The prior temperature history did not change the browning reaction rate constant at other temperatures.

CONCLUSIONS

ANALYSIS of the kinetic data suggested a pseudo-first order reaction with an Arrhenius-type dependence on temperature. Furthermore, it may be inferred from the experiments that the NEB reaction rate depended not only on environmental conditions, such as temperature and water content, but also on the chemical composition and more critically on the total amino acids.

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Rheology of Apple Sauce: Effect of Apple Cultivar, Firmness, and Processing Parameters

M. A. RAO, H. J. COOLEY, J. N. NOGUEIRA, and M. R. McLELLAN

ABSTRACT

Shear rate-shear stress data of 54 samples of apple sauce and their serums were determined, and power law parameters were derived for the data. The samples were from two apple cultivars (Rome Beauty and Rhode Island Greening) that were stored to three firmness values and processed utilizing three finisher screen sizes and speeds. Serum samples were mildly non-Newtonian, while the sauce samples were highly non-Newtonian fluids. Analysis of variance showed that apple cultivar, apple firmness and finisher screen opening accounted for a large variation in sauce consistency, while regression analysis showed that pulp content was important for the magnitude of the sauce consistency.

INTRODUCTION

RHEOLOGICAL PROPERTIES of fluid foods are useful for designing flow and handling systems, quality control and for sensory evaluation (Sherman, 1970; Rao, 1977, 1986). Purees of fruits and vegetables are important items of commerce, and their flow properties have been studied extensively (Saravacos, 1968; Harper and Leberman, 1962; Barbosa Canovas and Peleg, 1983; Rao et al., 1974, 1981; Duran and Costell, 1982). The studies have been conducted with rotational and tube viscometers. In most of the studies, the shear rate-shear stress data were utilized to derive parameters of the simple power law model:

$$\tau = K \dot{\gamma}^n \quad (1)$$

The magnitude of the flow behavior index n is less than 1 for shear-thinning (pseudoplastic) fluids, greater than 1 for shear-thickening (dilatant) fluids, and equal to 1 for Newtonian fluids. The consistency index is equal to the viscosity when n equals 1.

Barbosa Canovas and Peleg (1983) also studied the applicability of the Casson (Eq. 2), Herschel-Bulkley (Eq. 3), and the Mizrahi-Berk (Eq. 4) models to commercial samples of baby foods, apple sauce, and mustard.

$$\tau^{0.5} - K_{OC} = K_C \dot{\gamma}^{0.5} \quad (2)$$

$$\tau - \tau_{OH} = K_H \dot{\gamma}^{n_H} \quad (3)$$

$$\tau^{0.5} - K_{OM} = K_M \dot{\gamma}^{n_M} \quad (4)$$

The applicability of the same models to tomato concentrates was studied by Rao et al. (1981) and Rao and Cooley (1983). The Casson model (Eq. 2) predicts magnitudes of yield stress (K_{OC}^2) higher than those predicted by the Herschel-Bulkley (Eq. 3) or experimentally determined values (Vitali and Rao, 1984a).

There are several steps in the preparation of fruit and vegetable purees: peeling of the skin of the fruit, size reduction, heating to either soften the tissue and/or to inactivate enzymes, straining of the heated mass through finishers (finishing), and the addition of starch or sugar to obtain the desired consistency. The finishing operation (screen size and finisher speed) can be expected to have significant influence on the consistency of the sauce due to its effect on the quantity and the dimensions of the pulp.

In commercial practice, instruments such as the Bostwick Consistometer and the Adams Consistometer (USDA Flowsheet) are employed for quality control of apple sauce and other pureed foods. The Bostwick and Adams consistencies are empirical quantities that have proved to be useful for quality control purposes. However, they are not based on fundamental flow considerations and their magnitudes are not in terms of fundamental units of mass, length and time. In addition, there are limits to their use with pureed products. For example, the magnitude of Bostwick consistency decreases exponentially with increase in total solids of a pureed food (Marsh et al., 1978) so that reliable Bostwick consistency data of purees with more than about 17% total solids cannot be obtained; also, it is difficult to study the effect of temperature on the consistency of purees due to the lack of adequate temperature control. In contrast, rheological data in terms of shear rate-shear stress with a well designed viscometer permit taking into consideration the effect of temperature and concentration in a quantitative manner. The parameters so determined can be employed for many engineering applications (Rao and Anantheswaran, 1982).

Drake et al. (1979) and Drake and Spayd (1982) studied the influence of controlled atmosphere storage and peeling treatment on apple sauce quality. They measured the consistency of the sauce with an USDA Flow Sheet and the viscosity with a Brookfield viscometer.

The effect of different apple cultivars and magnitudes of firmness of apples on the flow properties of apple sauce determined with a viscometer capable of taking into consideration the non-Newtonian nature of apple sauce would be of interest to commercial producers of the product. Also of interest would be the effect of changing the speed and screen size of the finisher on the flow properties. The relative importance of soluble solids (sugars and pectic substances) and insoluble matter (pulp), as well as that of the particle size of the insoluble matter would be of interest to food technologists and to food rheologists. These aspects, which were not previously reported, were the objectives of the present study.

MATERIALS & METHODS

Apple sauce samples

Fifty four 18 kg applesauce samples were prepared in our pilot plant using Rome and R.I. Greening apples having three raw firmness values: 20, 15, and 10 lb-force. The apples were harvested from the Experiment Station Orchards when their firmness value was 20 lb-force and stored at 0°C to obtain the firmness values: 15 and 10 lb-

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force. The firmness of the apples was measured with an Effegi Fruit Tester (Model FT 327, 0-27 lb-force). The apples were processed using a pilot scale applesauce cooker as described by LaBelle et al. (1960) with the following modifications. The samples were prepared using three finisher speeds (rpm): 500, 700, and 900, and three finisher screen sizes (inches): 1/8, 3/8, and 1/16. The soluble solids of the sauce samples was adjusted to 16° Brix ± 1°. The Bostwick consistency of the samples was 4.6.

Rheological characterization

Rheological properties of the sauce samples were determined at 25°C with a concentric cylinder viscometer (Haake RV2, System MVI) over a common range of shear rates. The viscometer and the procedures for calculating the shear rate and shear stress were described earlier (Vitali and Rao, 1984a, b). Briefly, torque data were obtained at discrete values of rpm first in the ascending order followed by the descending order. Temperature of the samples was controlled with the aid of a constant temperature circulator (Lauda, Model K-2/R). Suspended solids were removed by centrifugation (10,000 × *g*) and the rheological properties of the serum were determined (Haake RV2, System NV). The effect of temperature (20–70°C) on the rheological properties was studied on three sauce and three serum samples made from Rome apples.

The simple power law model (Eq. 1) was employed to describe the shear rate-shear stress data of the sauce and serum samples as described earlier (Vitali and Rao, 1984a). This model was chosen because of its simplicity and because its parameters can be determined with relative ease for the large number of samples employed in this study.

Physical and chemical analysis

Particle size distribution of the suspended solids was determined by wet sieving (Nogueira et al., 1985). The pulp content was determined by centrifuging (3600 × *g*) samples diluted to 10° Brix (with distilled water) for 10 min; this procedure is similar to that employed for determining the pulp content of concentrated orange juice samples (Praschan, 1981). The pectin content was determined by chemical analysis as described by Kintner and Van Buren (1982).

Using a statistics computer program (GENSTAT, Rothamsted Experiment Station), nonlinear regression analyses were performed with the apparent viscosities of serum and sauce at a shear rate of 100 sec^{-1} as the dependent variables, and with the composition parameters as the independent variables. Analysis of variance (ANOVA) was also performed using the computer program GENSTAT to determine the influence of the cultivars, fruit firmness, and finisher speed and screen size on the rheological properties.

RESULTS & DISCUSSION

Physical model of apple sauce

Apple sauce can be viewed as consisting of suspended pulp in serum. The rheological properties of the serum wil depend on the soluble solids. In the case of the sauce, the rheological properties will depend not only on those of the serum but on the quantity of the pulp and its charcteristics such as the dimensions of the pulp particles. In the following, the various facets of this model will be explored. Pulp content is probably a new concept in the case of apple sauce whose role in the rheological properties needs to be studied. In the case of concentrated orange juice it is used as a quality parameter and its influence on rheological properties has been well demonstrated (Mizrahi and Berk, 1972; Vitali and Rao, 1984a).

In general, the types of data analyses employed here will be applicable to other pureed foods and apple sauce samples. However, the magnitudes of coefficients in the mathematical relationships will not be applicable to other apple sauce samples and pureed foods. This is due to the variation inherent to horticultural products in terms of cultivars and seasonal variations.

Rheological parameters of sauce and serum

Because of time dependence, power law parameters in the ascending order of shear rates were different from those obtained in the descending order. In general, the consistency index of the ascending order were higher than those in the descending order but the flow behavior indexes were not significantly different. Barbosa Canovas and Peleg (1983) also observed time dependent behavior for apple sauce and other pureed foods. Because the time dependency was over a relatively short time period, only the steady state data were analyzed.

Table 1—Power law parameters[a] of apple sauce and serum

Sample[b]	n (–)	K (N sec^n/m^2)	R
	Apple sauce		
R11	0.314	16.45	0.999
R12	0.325	14.97	0.999
R38	0.399	8.03	0.999
R39	0.381	9.92	0.999
G11	0.297	19.95	0.999
G12	0.316	16.13	0.999
G38	0.397	7.38	1.000
G39	0.349	12.29	0.999
	Apple sauce serum		
R11	0.753	0.064	0.999
R12	0.770	0.063	0.999
R38	0.857	0.018	1.000
R39	0.838	0.022	1.000
G11	0.770	0.138	0.998
G12	0.767	0.139	0.998
G38	0.808	0.041	0.997
G39	0.812	0.044	0.997

[a] Power law parameters: n is flow behavior index and K is the consistency index.
[b] Sample code: R for Rome, G for Greening; first numeral refers to firmness (1 for 20 lbf, 2 for 15 lbf, and 3 for 10 lbf), and last numeral refers to finisher speed-screen combination (1 for 900 × 0.012, 2 for 900 × 0.09, 3 for 900 × 0.06, 4 for 700 × 0.012, etc.).

For illustrative purposes, the power law parameters and the corresponding correlation coefficients of a few sauce samples are given in Table 1. The correlation coefficients indicate that the power law model described well the shear rate-shear stress data. Overall, the magnitudes of the power law parameters are consistent with those reported in the literature (Saravacos, 1968; Barbosa Canovas and Peleg, 1983). It is of interest to note that even though the sauce samples were adjusted to a constant Bostwick consistency when hot, the magnitudes of the power law consistency index varied over a wide range. This observation points out one limitation of Bostwick consistency for use as a rheological parameter of pureed foods.

Table 1 also contains the power law parameters and the corresponding correlation coefficients of a few serum samples. Again, the power law described well the rheological data. The serum samples were pseudoplastic and mildly non-Newtonian due to the presence of dissolved pectins. In contrast, the sauce samples were highly non-Newtonian (Table 1) due to the additional effect of suspended matter. The consistency index of sauce (CISA) was higher than that of serum again due to the suspended matter. These results are in qualitative agreement with those for concentrated orange juice and its serum (Mizrahi and Berk, 1972; Vitali and Rao, 1984b).

Effect of temperature on apparent viscosity. The activation energy of flow (E_a) of three sauce samples made from Rome apples was determined from the Arrhenius equation using the apparent viscosity at a shear rate of 100 sec^{-1}:

$$\eta_a = \eta\infty \exp (E_a/RT) \quad (5)$$

The apparent viscosity was calculated from the relation:

$$\eta_a = K \dot{\gamma}^{n-1} \quad (6)$$

The magnitude of E_a for the sauce samples were between 4.3–4.5 kcal/mole, while those for the serum samples were 5.5–6.2 kcal/mole. The lower magnitudes of E_a for the sauce samples were due to the presence of insoluble matter (pulp). Vitali and Rao (1984b) also noted that the magnitudes of E_a for concentrated orange juice samples were lower than those for the corresponding serum samples.

Correlation between consistency and flow behavior in-

Table 2—Analysis of variance of the consistency index (K) of apple sauce

Source of variation	DF[a]	MS[b]	F-Statistic
Cultivar	1	198.6	58.3**
Firmness	2	344.7	101.2**
Finisher speed	2	8.9	2.62
Finisher screen size	2	30.5	8.96**
Cultivar × firmness	2	26.8	7.85**
Residual	44	3.4	

[a] DF means degrees of freedom.
[b] MS is mean square.
** Significance at P>0.99.

dexes. Linear regression analysis indicated that the consistency and flow behavior indexes of the sauce FBSA were highly correlated negatively ($R^2 = 0.947$):

$$CISA = 52.2 - 111.7\ FBSA \qquad (7)$$

This relation points out that in general, the consistency index was higher for the more pseudoplastic samples. The upper limit for the magnitude of CISA was 52.2 and that samples with magnitudes of flow behavior index of 0.467 or higher would have negligible magnitudes of CISA. It is emphasized that the coefficients in Eq. (7) are valid only for the samples of the present study, but the concepts of negative correlation between FBSA and CISA as well as the discussion regarding the limiting values should be valid for other pureed foods. However, for a given product, relationships such as Eq. (7) should not be used for ranges of variables beyond those employed; in particular, negative values of consistency index will not be meaningful.

Influence of storage and processing parameters

Consistency index of sauce (CISA). The mean value of CISA of Greening apples was higher than that of Rome apples; the magnitudes were 14.3 and 10.5 N sec^n/m^2, respectively. The mean values for firmness stratum were: 17.2, 11.4, and 8.6 N sec^n/m^2, for apple firmnesses of 20, 15, and 10 lbf, respectively. Analysis of variance (Table 2) indicated that the effects of cultivar and firmness as well as the interaction of cultivar and firmness on CISA were highly significant (P>0.99). The influence of finisher screen size on CISA was also highly significant (P>0.99), but that of the finisher speed was not significant. The magnitude of CISA increased with decrease in the screen size from 0.09 in. to 0.06 in., but it did not change when screen size was changed from 0.12 in. to 0.09 in.; the mean values were 11.4, 12.0, and 13.9 N sec^n/m^2 for the screen sizes of 0.12, 0.09, and 0.06 in., respectively. The interaction effects of the other variables were not significant and, hence, they were not included in Table 2.

Flow behavior index of sauce (FBSA). Overall, sauces from Greening apples were more pseudoplastic than those from Rome apples. The mean values of FBSA were 0.340 and 0.373 for the Greening and Rome apples, respectively. Apple sauce samples made from firm apples were more pseudoplastic than those from soft apples. The magnitudes of mean values of FBSA were 0.315, 0.364, and 0.390, for firmness magnitudes of 20, 15, and 10 lbf, respectively. The influence of cultivar and firmness as well as the interaction of cultivar and firmness on FBSA were highly significant (P>0.99) (Table 3). The magnitude of FBSA decreased (increase in pseudoplasticity) with decrease in finisher screen size. The mean values of FBSA were 0.364, 0.359, and 0.347 for screen sizes of 0.12, 0.09, and 0.05 in., respectively. The effect of screen size on FBSA was highly significant (P>0.99), while that of the finisher speed was significant (P>0.95). The interaction effects of the other variables were not significant and, hence, they were not included in Table 3.

It should be noted that the observations with regard to the magnitudes of FBSA and CISA are consistent with the earlier observation that they were negatively correlated.

Table 3—Analysis of variance of the flow behavior index (n) of apple sauce

Source of variation	DF[a]	MS[b]	F-Statistic
Cultivar	1	0.0150	69.2**
Firmness	2	0.0267	122.9**
Finisher speed	2	0.0009	4.3*
Finisher screen size	2	0.0014	6.4**
Cultivar × firmness	2	0.0032	14.7**
Residual	44	0.0002	

[a] DF means degree of freedom.
[b] MS is mean square.
*Significance at P>0.95.
**Significance at P>0.99.

Table 4—Correlation forms for serum apparent viscosity

Correlation 1:

$$\eta_{se,100} = A + B\,(PECT)^C \times (BRIX)^D$$

$A = 0.01387$, $B = 5.897 \times 10^{-7}$, $C = 5.238$, $D = 2.183$, $R^2 = 0.828$

Correlation 2:

$$\eta_{se,100} = A + B\,(PECT \times BRIX)^C$$

$A = 0.0118$, $B = 2.271 \times 10^{-9}$, $C = 4.463$, $R^2 = 0.827$

Correlation 3:

$$\eta_{se,100} = A + B\,(PECT)^C$$

$A = 0.01369$, $B = 3.0474 \times 10^{-4}$, $C = 5.048$, $R^2 = 0.824$

Correlation between flow properties and composition

Correlations between the flow properties of the serum and sauce, and the composition variables will be useful for assessing the relative importance of the components. Based on the earlier model of apple sauce, rheological properties of serum will be dependent on the soluble solids in it, principally sugars and pectins. In the case of apple sauce, the rheological properties will be dependent on those of serum, and the amount of pulp and the average particle size of the pulp. Correlations were attempted with the apparent viscosity as the dependent variable. Consistency indexes were not used because their magnitudes reflect those of the flow behavior indexes. However, the correlation forms determined here should be applicable to consistency indexes also.

Correlation of apparent viscosity of serum. Different correlation forms were tested with the apparent viscosity of serum at a shear rate of $100sec^{-1}$ ($\eta_{se,100}$) as the dependent variable and the °Brix and pectin content (mg/mL) as independent variables. The correlation forms ranged from simple additive, exponential, power and sum of power terms. The square of the correlation coefficient (R^2) was used as the criterion for goodness of fit of a model to the data. The correlation forms with high R^2 are shown in Table 4. Because of the limited range in °Brix of the serum samples, its influence on $\eta_{se,100}$ was not significant, so that correlations based only on pectin content were nearly as good as those containing both °Brix and pectin content.

The additive form correlations are of particular interest because they yield finite magnitudes when the amount of pectin and sugar become zero. Ideally, the limiting value should reflect the magnitude of the aqueous solution of various dissolved salts and organic compounds. However, due to errors inherent in extrapolation procedures, they may not be representative of the magnitudes of the viscosities of dilute aqueous solutions.

The magnitudes of R^2 listed in Table 4 are not very high. Other correlation forms were tested, such as including the viscosity of 16.34 °Brix sucrose solution which represented an average value for all the samples. However, they resulted in lower magnitudes of R^2.

Correlation for apparent viscosity of sauce. As in the case of serum, different correlation forms were tested and the correlation with the highest R^2 (0.986) was:

$$\eta_{sa,100} = \eta_{se,100} + 6.09 \times 10^{-3}\,(PU)^{1.278}\,(PS)^{0.015} \qquad (8)$$

One feature of the above correlation is that it reduces to the apparent viscosity of the serum when the pulp content is zero.

The small magnitude of the exponent on average particle size indicated that the apparent viscosity of the studied sauce samples was not affected significantly by particle size.

The correlation form for the apparent viscosity of sauce should be applicable to other pureed foods. However, the magnitudes of the various coefficients presented here will not be applicable to other pureed foods. A practical application of the various correlations is that the relative importance of the various constituents can be estimated from them.

In fact, because of the small magnitude of the exponent (0.015), the contribution of the average particle size of the sauce is not as important as that of the pulp content. For this reason, the average particle size term can be omitted to obtain the simpler correlation:

$$\eta_{sa,100} = \eta_{se,100} + 6.423 \times 10^{-3} (PU)^{1.261} \quad (9)$$

In Eq. 9, the exponent (1.261) of the PU term and the constant (6.423×10^{-3}) are nearly equal to the corresponding terms in Eq. (8) confirming the small influence of the average particle size on η_{sa}.

The relative contributions of the pulp content and the apparent viscosity of the serum can be studied from Eq. (9). At first glance, because of the small magnitude of the coefficient (6.423×10^{-3}), it is tempting to conclude that pulp content does not play a major role on the viscosity of apple sauce. However, by equating the two terms on the right hand side of Eq. (9), one can determine the pulp content at which the contribution of the pulp to $\eta_{sa,100}$ equals that by the serum. For the mean value of $\eta_{se,100}$ of 0.0262 Pa. sec of all the samples, the equivalent pulp content was determined to be 3.05%. Because the pulp content of the sauce samples ranged between 30 and 45%, the contribution of pulp to the magnitude of sauce viscosity was much higher than that of the serum apparent viscosity.

CONCLUSION

THE CONSISTENCY and the flow behavior indexes of apple sauces were influenced significantly by the apple cultivar and firmness and the size of the finisher screen. The apparent viscosity and the sauce can be correlated in terms of the serum apparent viscosity and the sauces' pulp content. The pulp content played a major role in determining the magnitude of the apparent viscosity of the sauce; in contrast, the serum apparent viscosity and the average particle size of the pulp played minor roles.

NOMENCLATURE

A, B, C, D	Constants in correlating equations
CISA	Consistency index of sauce (N sec^n/m^2)
E_a	Activation energy of flow (kcal/mole)
FBSA	Flow behavior index of sauce (−)
K	Consistency index, power law model (N sec^n/m^2)
K_C, K_{OC}	Parameters in Casson model, Eq. (2)
K_H	Consistency index, Herschel-Bulkley model, Eq. (3)
K_M, K_{OM}	Parameters of Mizrahi-Berk model, Eq (4)
n, n_H, n_M	Flow behavior index (−)
PS	Average particle size of pulp (mm)
PU	Pulp content (volume %)
R	Gas constant (cal/mole °K)
T	Absolute temperature (°K)
Greek Letters	
$\dot{\gamma}$	Shear rate (sec^{-1})
η_a	Apparent viscosity (Pa sec)
η_∞	Constant in Arrhenius equation (Eq. 5)
τ	Shear stress (N/m^2)
Subscripts	
sa	sauce
se	serum

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Enzyme-Linked Immunosorbent Assay for Detection of Mold in Tomato Puree

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ABSTRACT

A double-sandwich enzyme-linked immunosorbent assay (ELISA) was developed for the detection of molds (*Alternaria alternata, Geotrichum candidum* and *Rhizopus stolonifer*) in tomato puree by the use of antisera raised in rabbits injected with homogenates of the lyophilized boiled molds. Cross reactivity among the three species was less than 10%. Detection limits were approximately 1 μg dried mold/g of sample, a sensitivity greater than that of most chemical methods. Positive relationships between ELISA readings and the amounts of mold added to puree were observed, while background ELISA values for puree controls were negligible.

INTRODUCTION

FUNGAL CONTAMINATION has been a recurrent problem in the tomato processing industry. Spoilage usually occurs after physical damage to ripened fruits in the field or processing plant. The presence of mold in thermoprocessed products indicates either unsanitary processing conditions or unacceptable raw ingredient quality. Generally the sanitation of the processing operation is assessed by using machinery mold (*Geotrichum candidum*) as an indicator. Raw ingredient quality is determined in part by the Defect Action Levels, which were established by the U.S. Federal Food and Drug Administration (FDA) to regulate natural or unavoidable defects in food that present no human health hazard. The official method currently being used by the FDA to determine the degree of fungal contamination in tomato and other food products is the Howard mold count (HMC) (Howard, 1911) which involves the microscopic counting of mold filaments. Recently, new processing techniques in the tomato industry have resulted in different degrees of product comminution which ultimately affect the HMC (Kallas, 1980). Methods based on the chemical or immunological detection of fungal components may provide more satisfactory alternatives for assessing fungal contamination.

Fungal antigens have been detected by immunological methods, such as precipitin tests, complement fixation tests and immunoelectrophoresis tests, especially in medical diagnosis (Pepys and Longbottom, 1978; Kaufman, 1980), but many of these tests are inconvenient to apply or insufficiently sensitive for detecting molds in food products. The development of enzyme-linked immunosorbent assay (ELISA) (Engvall and Perlmann, 1972) now offers a sensitive, quantitative alternative to conventional immunoassays. Although a wide variety of biological compounds, viruses and bacteria have been detected by ELISA (O'Sullivan, 1984), limited research has focused on the detection of fungi. Casper and Mendgen (1979) and Nachmias et al. (1979) used ELISA to detect the mycelium of *Verticillium lecanii* in wheat leaves and the fungal filtrate from *Phoma tracheiphila* in lemon, respectively. Later, the detection of *Epichloë typhina* in tall fescue was reported by Johnson et al. (1982). A survey of the literature revealed that the technique has not previously been used for the detection of molds in processed food products.

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The objective of this research was to develop a double-sandwich ELISA for the detection of three species of mold (*Alternaria alternata, G. candidum* and *Rhizopus stolonifer*) in tomato puree.

MATERIALS & METHODS

Mold preparation

The three molds used in this study were American Type Culture Collection (ATCC) species: *G. candidum* (ATCC 7115), *R. stolonifer* (ATCC 16985) and *A. alternata* (ATCC 34509). Dried mold preparations representing cultures from 1 to 10 days of age were prepared according to Cousin et al. (1984), except that cultures were heated at 100°C for 90 min before lyophilization, to simulate the thermoprocessing of foods.

Preparation of rot-free and molded tomato puree.

Visibly rot-free tomatoes and tomatoes inoculated with molds (incubated at 26°C for 10 days) were processed individually as described by Cousin et al. (1984), except that portions of molded puree were mixed with rot-free puree before canning.

Antisera production

Antisera to the three different species of molds were produced individually by injecting New Zealand rabbits once intramuscularly with 15 mg of buffered suspensions of dried mold emulsified in Freund's complete adjuvant (final volume of emulsion 3 mL), followed by similar booster injections (emulsified with Freund's incomplete adjuvant) given at the third and eighth week after the initial injection. Rabbits were bled at intervals between and after injections (Fig. 1). Antibody titers of the sera were compared by Ouchterlony double diffusion tests in agar (Ouchterlony, 1962) and by ELISA (Fig. 1).

ELISA procedure

A double-sandwich ELISA procedure (Voller et al., 1979) was used as follows:

Purification of immunoglobulin (Ig). Two milliliters of rabbit serum were diluted with 8 mL of distilled water and 10 mL of 36% Na_2SO_4 were added dropwise with stirring at room temperature. The precipitate was collected, dissolved in 2 mL phosphate buffered saline (PBS) and dialyzed three times against 500 mL PBS. Some Ig preparations were further purified by DEAE-Sephacel filtration (Clark and Adams, 1977). Concentrations of Ig were adjusted to approximately 1 mg/mL by diluting the preparations with PBS to an A_{280} of 1.4.

Conjugation of alkaline phosphatase with immunoglobulin. Five milligrams of alkaline phosphatase (Sigma, Type VII-T) and 2 mg of Ig were mixed together to a total volume of 3 mL and dialyzed three times against PBS. Glutaraldehyde was added to the dialyzed mixture to a final concentration of 0.06%. After a 4 hr reaction period at room temperature, the conjugate was again dialyzed against PBS. An equal volume of 1% bovine serum albumin (BSA) with 0.04% NaN_3 was then added to the conjugate, before storage at 4°C.

Assay procedure. Two hundred microliters of Ig diluted in a coating buffer of pH 9.6 (1.59g Na_2CO_3, 2.93g $NaHCO_3$, and 0.2g NaN_3 per liter of distilled water), were added to the wells of polystyrene microtiter plates (Immulon I, Dynatech Laboratories, Inc., 900 Slater Lane, Alexandria, VA), which were then incubated at 37°C for 2 hr and then washed three times with PBS-Tween (PBS with 0.05% Tween 20). To the Ig-coated wells, 200 μL of test sample (either mold suspension or mold-spiked tomato puree diluted fivefold in PBS-Tween) were added. Each sample was done in duplicate. Plates were then incubated at 37°C for 4 hr and washed as before. Then 200 μL of

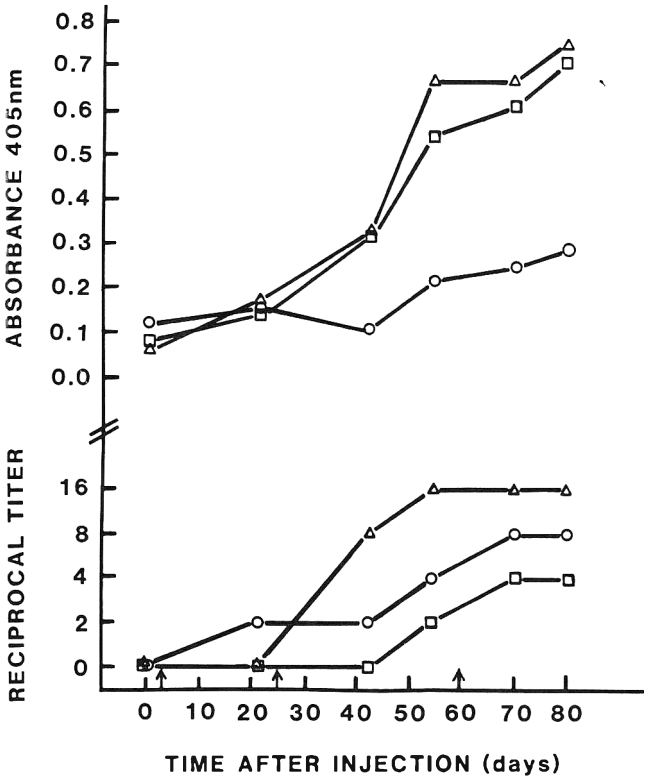

Fig. 1—Reciprocal titers in gel diffusion tests and ELISA absorbance values of rabbit antisera to 0.1 mg/mL each of G. candidum *(△),* A. alternata *(□) and* R. stolonifer *(○). The coat Ig/conjugate combinations were 10/1, 10/5, and 10/5 μg/mL, respectively. ↑ : Injection.*

alkaline phosphatase-Ig conjugate diluted in conjugate buffer (PBS-Tween with 0.2% ovalbumin) were added to each well, and the plates were incubated at 5°C overnight. The plates were then washed as before and 200 μL of 1 mg/mL p-nitrophenyl phosphate diluted in substrate buffer (pH 9.8, 97 mL diethanolamine, 0.2g NaN_3 per liter of distilled water) were added to each well and the plates incubated at room temperature for 35 min. Substrate reactions were stopped by adding 50 μL 3N NaOH and the A_{405} of each well was measured with an ELISA reader (Bio-Tek Instruments, Inc.).

RESULTS & DISCUSSION

Antisera production.

The antibody titers of rabbit bleeds were monitored by the Ouchterlony test (Ouchterlony, 1962). They increased slowly and reached a plateau after the second injection for *G. candidum*, and after the third injection for *R. stolonifer* and *A. alternata*, with final reciprocal titers of 16, 8 and 4, respectively (Fig. 1). Relatively low sensitivity of the Ouchterlony test with molds and their antisera was also indicated by Casper and Mendgen (1979) and Nachmias et al. (1979). In their studies, the reciprocal titers of antisera in Ouchterlony tests were 16 and 32 for *Verticillium lecanii* and *Phoma tracheiphila*, respectively. ELISA tests indicated similar overall trends in antibody titer, but with somewhat different sensitivity relationships among the bleeds (Fig. 1). For the last bleeds collected, antibody induced by *G. candidum* gave the highest ELISA readings, while those for *A. alternata* antibody were intermediate and those for *R. stolonifer* gave the lowest readings. Disparities between the Ouchterlony titers and sensitivities in ELISA may reflect the different binding reaction mechanisms involved (Lister et al., 1983). The antisera also reacted in Ouchterlony tests with extracts from the nonheated fresh or lyophilized molds, giving precipitation lines that joined without spurring with those produced with extracts from the heated preparations.

Detection of mold by ELISA

In comparative tests, the ELISA sensitivity for antigen detection was approximately ten thousand fold or greater than that of the Ouchterlony test, depending on the mold assayed. Different combinations of dilutions of coat Ig and conjugate Ig (Fig. 2, A-C) were compared for detecting the molds by ELISA. The optimal coat/conjugate concentrations for detecting *G. candidum, R. stolonifer*, and *A. alternata* were 1/1, 10/5, and 10/5 μg/mL, respectively. With these combinations, mold concentration and ELISA value ratios were essentially linear in the range 1 μg/mL to 1 mg/mL of mold. Detection limits were below 100 ng/mL, 1000 ng/mL, and 100 ng/mL for *G. candidum, R. stolonifer*, and *A. alternata*, respectively, assuming positive values were those exceeding twice the background (i.e., values for buffer controls). The background readings were very low, usually ranging from 0.01 to 0.02. In the study of Johnson et al. (1982), the detection limit for *Epichloë typhina* by ELISA was 100 ng/ml, which is similar to our results.

There was little or no cross reactivity among the three species (Table 1). The ratios of heterologous to homologous reactions between sera and molds were very low (<10%). Most reactivity resided in the PBS-soluble fraction of extracts, since when extracts were clarified by centrifugation at 8,000 × *g* to remove particulate material, ELISA readings were reduced by only 18 – 28% (Table 1).

Detection of mold added to rot-free tomato puree by ELISA

In comparisons of ELISA detection of molds diluted in PBS-Tween or in rot-free tomato puree, the effects of tomato puree on ELISA efficiency appeared to be relatively slight (Fig. 3, A-C). Background readings for unspiked tomato puree controls ranged from 0.01 to 0.05, depending on the mold species used, indicating negligible cross-reaction between the mold antisera and the plant materials. With *G. candidum* and *A. alternata* (Fig. 3A and 3C) the presence of tomato puree slightly reduced the sensitivity of mold detection by ELISA, but the reverse was true for *R. stolonifer* at lower concentrations (Fig. 3B). These effects are presumably due to nonantigenic components in tomato puree, which affect the binding of fungal antigen(s) and Ig.

Detection of molded puree by ELISA

Since molds growing in either artificial media or on fresh tomatoes may have different morphologies and, possibly, antigenicities, tomatoes were inoculated with molds, processed and assayed. Molded tomato puree was mixed in different concentrations with rot-free puree before canning and thermoprocessing. One part of molded puree per thousand or ten thousand parts of rot-free puree could be detected (Fig. 4) depending on the mold species. Therefore, whether molds grew on artificial media or on fresh tomatoes the efficiency of the assay remained the same.

Effects of modifications in the ELISA procedure

Clark and Adams (1977) included 2% polyvinylpyrrolidone (PVP) in the sample buffer to eliminate non-specific binding reactions of plant materials in ELISA. Also, to block unadsorbed sites on the solid phase, they included an additional blocking step of 1% bovine serum albumin (BSA; 37°C for 30 min) after the coating step (Clark and Adams, 1977). In the present experiments, the incorporation of 2% PVP into the mold sample buffer increased ELISA absorbances and also background readings (Table 2), while the use of a 1% BSA blocking step decreased both the sample and background ELISA readings (Table 2). Therefore, neither modification seemed advantageous.

The effects of incubation temperature and agitation were

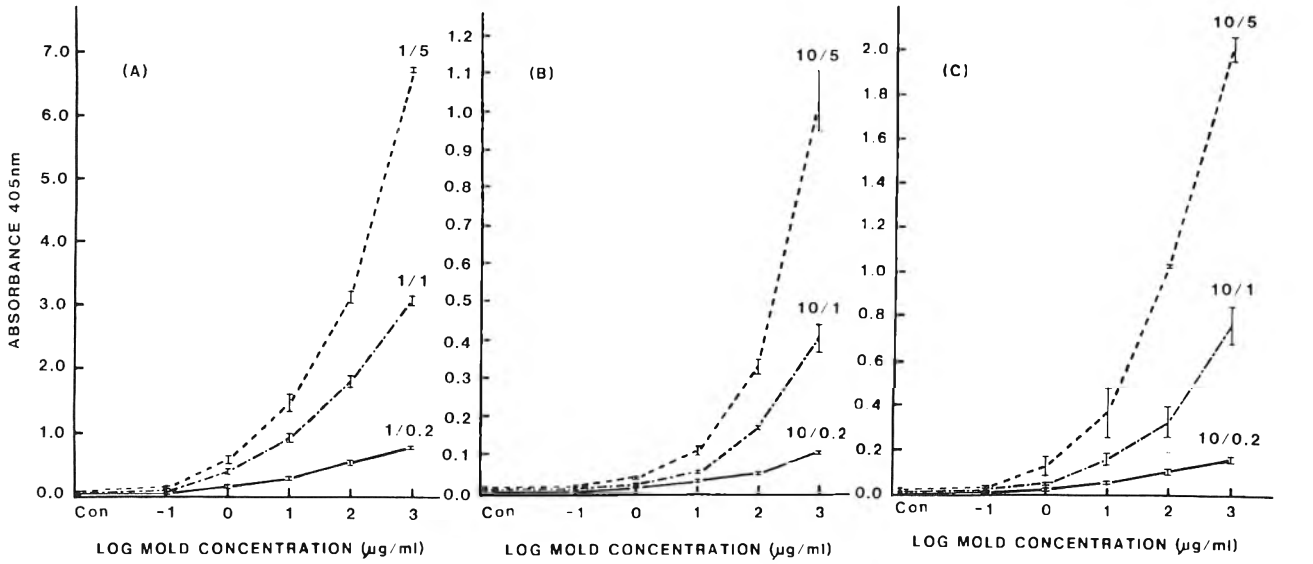

Fig. 2—ELISA absorbance values obtained with a range of mold concentrations, using different coat Ig/conjugate Ig (μg/mL) combinations, as indicated, for (A) G. candidum, *(B)* R. stolonifer, *and (C)* A. alternata. *Con: Control. The absorbances greater than 2 were extrapolated from dilutions.*

Table 1—Homologous ELISA absorbances obtained with PBS extracts of different species of molds before and after low speed centrifugation to remove particulate material[a]

Mold species	Antigen used	Ig used in ELISA		
		G. candidum	R. stolonifer	A. alternata
G. candidum	PBS extract[b]	0.929	0.001	0.010
	Centrifuged extract supernatant[c]	0.761	0.006	0.009
	% Difference[d]	18%	---	---
R. stolonifer	PBS extract	0.019	0.554	0.044
	Centrifuged extract supernatant	0.019	0.398	0.039
	% Difference	---	28%	---
A. alternata	PBS extract	0.013	0.020	1.303
	Centrifuged extract supernatant	0.015	0.023	1.024
	% Difference	---	---	21%

[a] The mold concentration was 1 mg/mL and the coat/conjugate combinations were 10/1, 10/5 and 10/5 (μg/mL) for *G. candidum, R. stolonifer* and *A. alternata*, respectively.
[b] Mold extract made by homogenization.
[c] Supernatants from preparations made as in "a," after low speed centrifugation.
[d] $\%\ \text{Difference} = \frac{A_{405}\text{ of PBS extract} - A_{405}\text{ of centrifuged supernatant}}{A_{405}\text{ of PBS extract}}$

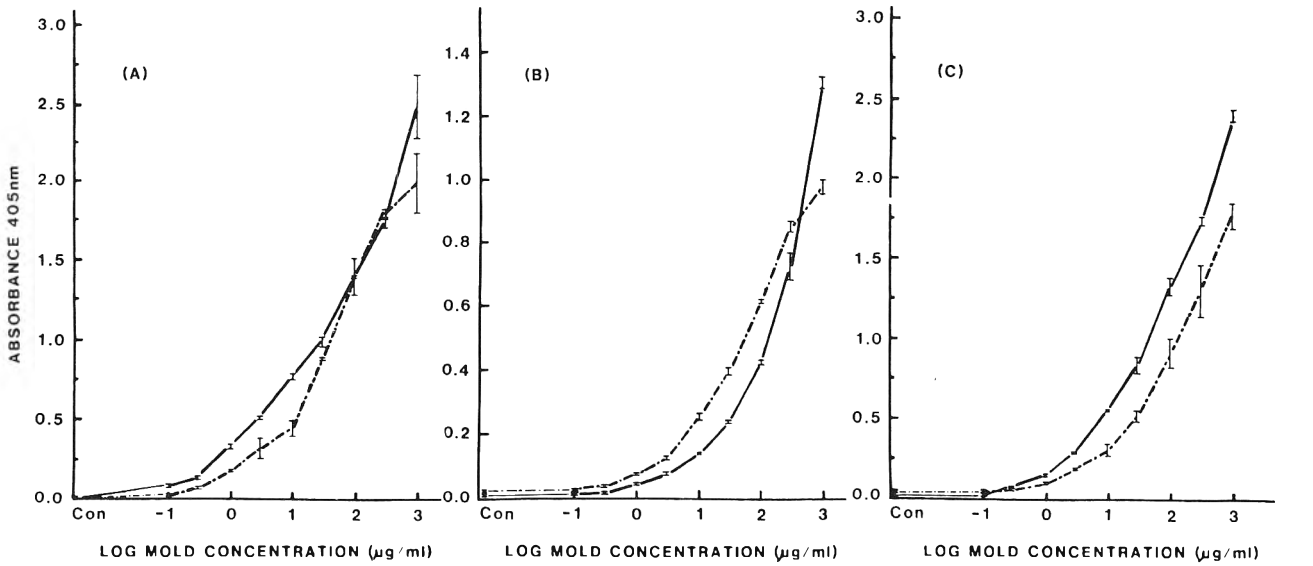

Fig. 3—ELISA absorbance values of various concentrations of mold extract of (A) G. candidum *(B)* R. stolonifer *and (C)* A. alternata *made in PBS-Tween (—) and mixed 1:5 (w/v) with rot-free tomato puree made in PBS-Tween (– –). The coat/conjugate combinations were 1/1, 10/5 and 10/5 μg/mL, respectively. Con: Control.*

Table 2—Effects on ELISA absorbances (A_{405}) of homologous and heterologous reactions of antisera and three mold species obtained after treatments including antigen (Ag) buffer with 2% PVP and use of a 1% BSA blocking step after coating with Ig[a]

Mold species	2% PVP in Ag buffer	Ig used in ELISA: *G. candidum* BSA blocking		*R. stolonifer* BSA blocking		*A. alternata* BSA blocking	
		with	without	with	without	with	without
G. candidum	with	0.814	1.159	0.059	0.070	0.061	0.086
	without	0.574	0.927	0.042	0.058	0.038	0.057
R. stolonifer	with	0.033	0.053	0.478	0.875	0.079	0.125
	without	0.019	0.033	0.372	0.618	0.051	0.067
A. alternata	with	0.023	0.037	0.053	0.072	0.740	1.081
	without	0.015	0.026	0.043	0.054	0.637	0.793
Tomato puree	with	0.027	0.040	0.056	0.069	0.058	0.084
Control	without	0.015	0.026	0.030	0.053	0.037	0.053

[a] The mold concentration was 1 mg/mL of a 1:5 (w/v) diluted rot-free tomato puree. The coat/conjugate combinations were the same as in Table 1.

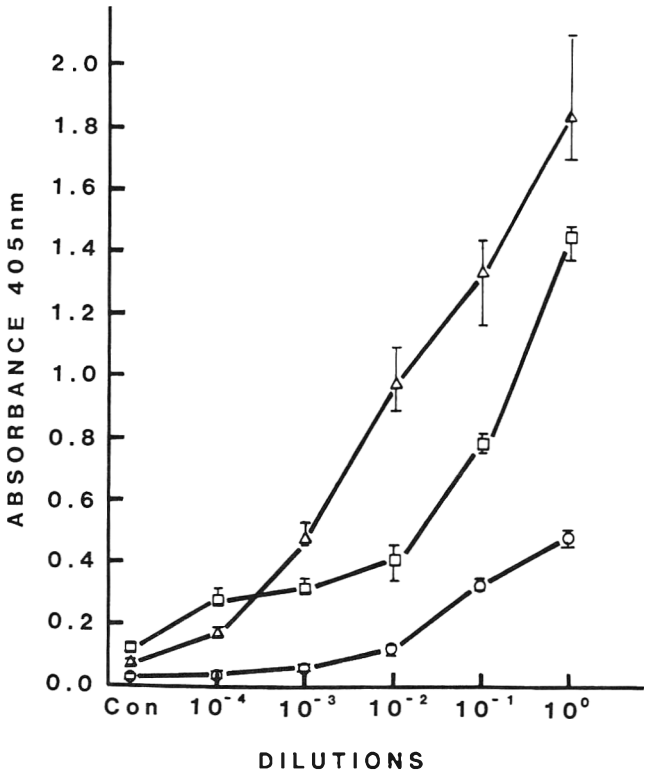

Fig. 4—ELISA absorbance values of molded tomato puree diluted with rot-free tomato puree for G. candidum *(△),* A. alternata *(□) and* R. stolonifer *(○). The coat/conjugate combinations were the same as in Fig. 3. Con:Control.*

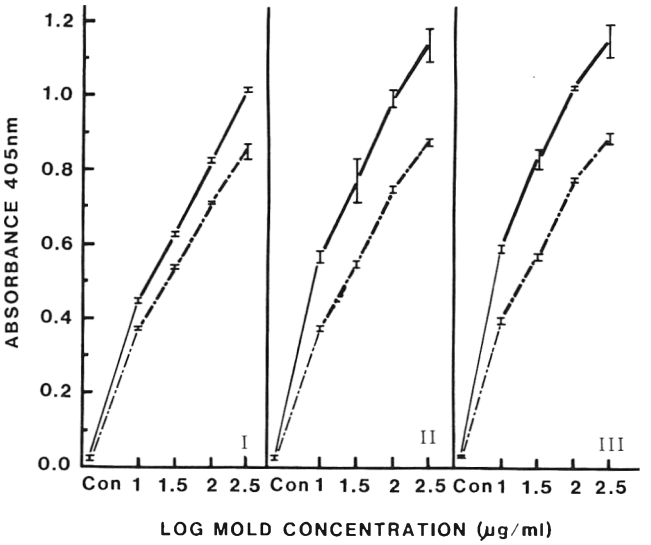

Fig. 5—ELISA absorbance values of G. candidum *(coat/conjugate = 10/1 μg/mL) made in PBS-Tween (—) and mixed 1:5 (w/v) with rot-free tomato puree made in PBS-Tween (– –) at different incubation temperatures and with or without agitation. I: 37°C, without agitation; II: room temperature, without agitation; III: room temperature, with agitation; Con: Control. Similar results were obtained for* R. stolonifer *and* A. alternata.

also investigated. Incubation at room temperature instead of 37°C somewhat increased the absorbance readings for mold extracts in PBS-Tween (Fig. 5), but for molds in tomato puree, the readings either remained the same or decreased (Fig. 5). These results indicated that the fungal antigen(s) involved reacted more efficiently with Ig at room temperature, but that this effect could be masked by the presence of tomato puree materials. Agitating the plate did not affect the readings (Fig. 5).

Because optimization of the ELISA procedure will depend upon the characteristics of the antisera used, the effects of variations in the timing of steps in the method were not tested exhaustively. However, reducing the total of the coating, sample incubation and conjugate reaction steps to 4 hr or less substantially reduced reaction sensitivity, especially in the presence of tomato puree (data not shown), suggesting that extended incubation times may be important for efficient detection of mold in such a product.

In tests of an indirect ELISA procedure, in which the test antigens were used to coat the plate prior to adding rabbit Ig followed by anti-rabbit Ig-enzyme conjugate, the results were inconsistent and correlations between mold concentration and ELISA absorbance were poor (data not shown). Presumably, adsorption of the fungal antigen to the plate during the coating step was not as efficient as the specific trapping of antigen on plates previously coated with their homologous Ig's.

Detection of mold in other fruit products

Although only *A. alternata*, *G. candidum*, and *R. stolonifer* were studied and detected in tomato puree samples, similar procedures could probably be used to detect these and other genera and species of mold in tomato puree and in other processed food products. Current research shows that these molds can also be detected in other fruit products, such as applesauce, peach puree, and apricot nectar (to be published). For screening food samples containing mixed populations of different species of mold, either an antibody against a certain indicator mold or an antibody "cocktail" containing a combination of various antibodies against several mold species could be produced and used. Further work (to be published) has indicated that the antigenic components, which reacted with periodic acid-Schiff reagent, are possibly polysaccharide in nature. They have molecular weights in the order of one million, according to estimates from Sepharose 6B gel filtration. No typical virus structures were observed in the high molecular weight antigens by electron microscopy, thus excluding the possibility that the antigens were virus-like particles such as are common in fungi.

—Continued on page 192

Effect of Initial Dissolved Oxygen Levels on the Degradation of Ascorbic Acid and the Browning of Lemon Juice during Storage

G. L. ROBERTSON and C.M.L. SAMANIEGO

ABSTRACT

The effect of different initial dissolved oxygen concentrations (0.41, 1.44, and 3.74 mg/L) on rate of deteriorative quality changes [ascorbic acid degradation, browning, hydroxymethylfurfural (HMF) and furfural production) in lemon juice stored at 36°C was investigated. No significant effects on rate of ascorbic acid degradation and furfural formation could be attributed to the different oxygen levels. Degradation of ascorbic acid appeared to be predominantly anaerobic. Lag period before browning increased depended on oxygen level. Zero-, first- and second-order kinetic models were fitted to various degradative reactions occurring in lemon juice during storage. Highly significant correlations were obtained between browning index, HMF, and furfural formation, suggesting that all three would be suitable as chemical indices of storage temperature abuse in lemon juices.

INTRODUCTION

DURING STORAGE of thermally preserved citrus products, many changes may occur that lead to a deterioration in quality. The extent of these changes depends on the processing technology, the quality of the raw material and packaging materials, and the storage conditions of the products. Two of the major changes are development of off-flavors (Tatum et al., 1975) and browning (Robertson and Reeves, 1981). This paper is concerned primarily with the latter change.

Various theories have been proposed to explain the mechanism of browning in citrus products (Shaw et al., 1977). In lemon juice, ascorbic acid degradation is thought to be the major mechanism since browning has been shown to be proportional to the level of ascorbic acid (Clegg, 1964). Because of the high acidity in lemon juice, it is considered unlikely that browning results from sugar-amino acid condensation. However, the presence of amino acids in ascorbate systems is a major contributor to the development of browning (Clegg, 1966).

The kinetics of the oxidative breakdown of ascorbic acid in liquid foods are complex. In addition to being dependent on light, pH and trace metals, the rate of ascorbic acid oxidation appears to be dependent on the dissolved oxygen concentration. Working with model solutions, where gas mixtures were bubbled through the solutions, Khan and Martell (1967) and Weissberger et al. (1943) indicated that the ascorbic acid destruction rates were directly proportional to the initial concentration of dissolved oxygen. However, caution must be used in drawing conclusions from such work and applying them to liquid foods since the experimental conditions used may not be directly applicable to liquid food systems.

Kefford et al. (1959) concluded that complete removal of oxygen from orange juice improved the retention of ascorbic acid and flavor during processing but had little effect on the retention of these quality factors during storage in metal cans. Johnson and Toledo (1975) studied ascorbic acid and color changes in orange juice concentrate. With atmospheric oxygen in the headspace the rate of browning and ascorbic acid degradation was rapid, the rate being retarded when headspace oxygen was reduced. Eliminating oxygen completely by storage in glass containers with zero headspace considerably reduced the rate of ascorbic acid degradation, 70% still being present after 10 wk at 24°C.

Burton et al. (1970) found that the stability of ascorbic acid was wholly determined by the level of residual oxygen in sterilized milk and not by the method of processing. When the oxygen content was low, ascorbic acid was stable indefinitely. Singh et al. (1976) working with liquid infant formula, concluded that when dissolved oxygen was maintained at saturation levels (8.71 mg/L), oxidative breakdown of ascorbic acid followed a first order mechanism. However, with limited oxygen, the reaction followed second order kinetics. In an associated study using the same material and methods (Mack et al., 1976), the rate of oxygen uptake from the headspace as a function of initial concentration of dissolved oxygen was investigated over 24 hr at three different initial concentrations. In the presence of light, the rate of oxygen uptake was found to be proportional to the initial concentration of dissolved oxygen. However, in the dark there were no significant differences in the rates. Ascorbic acid assays for the same samples indicated that the accelerated oxygen uptake rate was accompanied by only a slight increase in the rate of ascorbic acid loss (Mack, 1974).

Mannheim and Passy (1979) compared the effects of three methods of deaeration of single strength orange and grapefruit juices on ascorbic acid retention and browning. The effect of dissolved oxygen concentration on the rates of these reactions could not be evaluated since no measurements of dissolved oxygen were made. A study on grapefruit concentrate by Passy and Mannheim (1979) showed that deaeration treatments had no effect on quality. They concluded that oxygen consumed or lost from the headspace may account for only 2–3% of degraded ascorbic acid, and therefore the effect of oxygen was minimal and degradation was mainly anaerobic.

Eison-Perchonok and Downes (1982) studied ascorbic acid autoxidation by varying temperature, ascorbic acid and dissolved oxygen concentration. They calculated the dissolved oxygen concentration in their gas mixture experiments using a coefficient derived from Henry's Law. In the only check on the validity of their calculations with an oxygen probe, they obtained a dissolved oxygen concentration 42% greater than that calculated. For unspecified technical reasons, they could not check the other calculations but obviously their data must be approached with caution. They concluded that ascorbic acid autoxidation was dependent on the dissolved oxygen concentration.

In commercial practice citrus juices are deaerated and/or hot-filled into gas-impermeable containers. This reduces the dissolved oxygen concentration in the juice and the oxygen in the headspace. Hence, in commercial packs of citrus juice, oxygen availability is limited.

This study was undertaken to determine the effect of different initial dissolved oxygen concentrations on quality changes in lemon juice during storage. The kinetics of the deteriorative quality reactions taking place during storage (ascorbic acid degradation, browning, and furfural and HMF formation) were also investigated.

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MATERIALS & METHODS

Juice samples

Freshly-extracted lemon juice was pasteurized (80°C; 30 sec) and processed immediately as described below. To obtain three different levels of dissolved oxygen in the juice, the juice was divided into three equal volumes (approximately 5 L each) and treated in the following way: The first batch (I) was bubbled with nitrogen gas for 15 min, the second batch (II) was deaerated by passing through a vacuum chamber (31 kPa absolute), and the third batch (III) was mixed vigorously and then bubbled with air for 20 min. The juices were filled into 250 mL flasks immediately after treatment and tightly covered with aluminium foil-lined rubber stoppers. The juice temperature was kept at 36 ± 0.7°C during treatment and filling. No headspace was allowed to remain in the flasks. The initial amount of dissolved oxygen measured for each batch was: I - 0.41 mg/L; II - 1.44 mg/L, and III - 3.74 mg/L.

All flasks were stored in a dark room whose temperature was controlled at 36°C. Sampling and analyses were conducted three days after storage and every 7 days thereafter for a period of 6 wk. Duplicate flasks were analyzed for each treatment.

Analysis

Total soluble solids and acidity. Total soluble solids (°Brix), percent titratable acidity, pH, and the Brix:acid ratio were determined by official analytical methods (AOAC, 1980).

Ascorbic acid. The AOAC method (1980) for determination of ascorbic acid content, using 2,6-dichlorophenolindophenol, was followed except for the end-point determination where the potentiometric method of Spaeth et al. (1962) was used. This method is more precise and reproducible than the colorimetric AOAC method, which is subject to error in end-point recognition, particularly for colored fruit juices (Nagy and Smoot, 1977). Replicate determinations in lemon juice gave a precision of ±0.2%.

Browning. Browning in the juices was measured using the method of Meydav et al. (1977). The browning index of the clarified samples was taken as the absorbance at 420 nm as measured in a Spectronic 70 spectrophotomer (Bausch and Lomb Inc.). Replicate determinations in lemon juice gave a precision of ±3.5%.

Furfural and hydroxymethylfurfural (HMF). Furfural was determined using the improved method of Dinsmore and Nagy (1974), based on the aniline-acetic acid reaction with furfural. HMF was determined by the colorimetric method of Meydav and Berk (1978) which is based on the thiobarbituric acid (TBA) color reaction with HMF. Stilling and Browning (1940) showed that HMF does not interfere with the determination of furfural using the aniline-acetic acid method. Replicate determinations in lemon juice of furfural and HMF gave a precision of ±3.4% and ±5.5%, respectively.

Dissolved oxygen. Dissolved oxygen in the juice was measured using a YSI model 54 ARC dissolved oxygen meter fitted with a YSI 5739 dissolved oxygen probe (Yellow Springs Instrument Co., Inc., Ohio, USA). The probe was inserted into the 250 mL flask filled with lemon juice until the rubber stopper around the probe made a firm seal with the neck of the flask. Stirring at a constant speed was accomplished using a magnetic stirrer. A few minutes were allowed for the probe to stabilize to the sample temperature before the dissolved oxygen content was measured. Replicate determinations in lemon juice gave a precision of ±6.6%.

Data analysis

The reaction order of the different quality parameters measured was determined graphically and by computation. A regression analysis was performed to determine the rate constants of the different reactions.

An analysis of covariance (ANACOVA) was performed to determine if significant differences existed among the samples due to the three treatments. This was performed using the Biomedical Computer Program (BMDP) 1V run on a PRIME 750 computer.

RESULTS & DISCUSSION

Acidity and total soluble solids

The lemon juice had an initial pH of 2.6, total soluble solids of 9.4° Brix, titratable acidity of 5.4% and a Brix:acid ratio of 1.75. No significant changes were detected in these parameters for the three treatments as expected. In a study on pH values and citric acid content of grapefruit juice samples stored at 50°C for 12 wk. Smoot and Nagy (1980) concluded that the acid environment of the juices does not change during storage.

Dissolved oxygen

There was a rapid disappearance of dissolved oxygen in the juice samples as shown in Table 1. After 7 days storage the dissolved oxygen content had reached almost the same low level in all three juices and remained relatively constant during the subsequent storage period. Although a similar trend was observed by Passy and Mannheim (1979) for the disappearance of oxygen from the headspace of canned grapefruit juice concentrate, the two situations are not comparable since oxygen participates in corrosion reactions in cans (Mannheim and Passy, 1979).

Ascorbic acid

Ascorbic acid concentration decreased with storage time from an initial concentration of 476 mg/L (Table 1) and a first-order reaction fitted the data better than a zero-order reaction (Table 2). Numerous studies have shown ascorbic acid degradation to be first-order (Huelin, 1953; Waletzko and Labuza, 1976; Saguy et al., 1978a; Passy and Mannheim, 1979).

However, Singh et al. (1976) and Lin and Agalloco (1979) reported that the first-order rate is valid only if the oxygen is present in abundance (for aerobic degradation) or if it is totally excluded (for anaerobic degradation). In instances where oxygen is present in a limited amount, such as in this experiment, second-order kinetics is followed, i.e. the reaction depends on both the oxygen and ascorbic acid concentrations.

Therefore, the results were also fitted to second-order reaction models. For the model where the rate was proportional to the concentration of ascorbic acid and oxygen, a poor fit was obtained. This was not unexpected since ascorbic acid degradation is likely to have become independent of oxygen concentration after 7 days when oxygen remained relatively constant at a very low level (0.013 mM compared to ascorbic acid at 2.7 mM). Under these conditions where one component is in excess the second-order reaction model reduces to a pseudo-first order model. However, for the model where the rate was proportional to the square of the ascorbic acid concentration,

Table 1—Changes in ascorbic acid (AA), browning index (BI), hydroxymethylfurfural (HMF) and furfural in lemon juice stored at 36°C

Dissolved O_2(mg/L)	Days	AA[a] (%)	BI (abs)	HMF (mg/L)	Furfural (mg/L)
0.41	0	100.0	0.138	1.31	0.09
0.12	3	93.2	ND[b]	1.42	0.32
0.12	7	74.5	0.134	1.48	0.87
0.14	14	63.5	0.141	1.64	1.35
0.14	21	58.0	0.148	2.49	2.54
0.14	28	50.3	0.172	3.10	3.21
0.12	35	51.2	0.186	3.52	4.87
0.12	42	50.5	0.189	4.51	5.31
1.44	0	100.0	0.138	1.31	0.09
0.22	3	91.9	ND	1.40	0.32
0.15	7	78.5	0.130	1.46	0.86
0.15	14	60.3	0.151	1.60	1.34
0.15	21	60.9	0.141	1.99	2.60
0.14	28	52.7	0.161	2.94	3.24
0.12	35	51.4	0.177	3.64	4.74
0.14	42	50.3	0.196	4.64	5.47
3.74	0	100.0	0.138	1.31	0.09
0.15	3	85.9	ND	1.56	0.32
0.12	7	73.0	0.148	1.72	0.89
0.12	14	56.8	0.150	1.78	1.43
0.12	21	56.0	0.160	2.42	2.69
0.14	28	46.0	0.181	3.42	3.53
0.12	35	46.4	0.199	4.02	5.09
0.14	42	47.1	0.200	5.09	5.77

[a] Initial ascorbic acid content = 476 mg/L

[b] ND = not determined

Table 2—Reaction orders and computed rate constants (based on regression analysis) for reactions occurring during storage of lemon juice

Parameter	Oxygen level (mg/L)	Zero-order k (conc days^{-1})	Zero-order C_o	Zero-order R^2 (%)	First-order k ($\times 10^{-2}$day^{-1})	First-order ln C_o	First-order R^2 (%)
Ascorbic acid	0.41	1.16	89.4	81.6	1.67	4.49	86.3
	1.44	1.15	89.8	82.8	1.65	4.50	87.3
	3.74	1.18	86.0	79.8	1.80	4.45	84.9
Browning (absorbance)	0.41	0.00191	0.14	94.2	1.16	−1.96	93.6
	1.44	0.00175	0.12	89.5	1.09	−2.11	89.8
	3.74	0.00163	0.13	94.6	0.97	−2.00	95.6
Furfural	0.41	0.130	−0.11	98.3	8.50	−1.35	84.0
	1.44	0.131	−0.12	98.7	8.53	−1.35	84.2
	3.74	0.140	−0.15	98.8	8.69	−1.34	84.2
Hydroxymethyl-furfural	0.41	0.075	1.03	95.4	3.05	0.218	97.7
	1.44	0.077	0.93	91.4	3.09	0.177	96.5
	3.74	0.086	1.05	94.4	3.18	0.275	97.0

R^2 values of 89.9, 91.1, and 88.4% were obtained for oxygen levels of 0.41, 1.44, and 3.74 mg/L, respectively.

These results suggest that anaerobic degradation was the major mode of ascorbic acid breakdown, and that the reaction at a temperature of 36°C and a storage period of 6 wk is best described by a second-order model where the rate is proportional to the square of the ascorbic acid concentration.

The results of the ANACOVA indicated no significant differences among the three treatments at a 5% probability level. This means that oxygen at the levels used does not significantly affect the rate of ascorbic acid degradation. This is not surprising considering the close agreement between the rate constants for the three treatments (2.48, 2.44 and 2.84 $\times$ 10^{-4}%$^{-1}$ day^{-1}, respectively) assuming the second order model discussed above. Singh et al. (1976) in their study of the effect of dissolved oxygen on ascorbic acid loss in infant formula, reported that no significant differences existed between the rate constants for samples with initial dissolved oxygen concentrations between 1.00 and 4.86 mg/L stored under dark conditions at 7.2°C.

The fact that the last three readings corresponding to 28–42 days did not vary greatly in any of the three juices, explains why the R^2 values in Table 1 are not as high for ascorbic acid as for the other quality parameters. The constant levels of ascorbic acid over the last 14 days of the experiment indicate that the reaction had reached equilibrium.

Browning

Browning is one of the main reasons for consumer rejection of citrus products and is more pronounced in light-colored citrus juices such as lemon juice. For the treatments with initial oxygen levels of 0.41 and 1.44 mg/L, there was a lag period where the browning measurement remained almost constant, prior to an increase in browning being observed. This initial lag, also observed by Karel and Nickerson (1984), was referred to as the time during which colorless intermediates of the browning reaction were formed (Clegg and Morton, 1965).

At the end of the lag period an increase in browning was observed which can be described by the following equation:

$$B = B_{lag} + k(t - t_{lag}) \quad (1)$$

where B_{lag} = browning at the start of the lag period, t = time in days, t_{lag} = time in days of lag period, and k = rate constant. The lag period was found to be 14 days when the initial oxygen concentration was 0.41 mg/L, 7 days when 1.44 mg/L and zero when 3.74 mg/L. Both a zero-order and a first-order model fitted the data equally well (Table 2).

Studies have reported browning in other citrus juices to be a zero-order reaction (Saguy et al., 1978b; Mannheim and Passy, 1979). Passy and Mannheim (1979) stated that the browning reaction is probably predominantly anaerobic, but noted that it is almost impossible to obtain a "free oxygen" environment to definitely confirm this.

Because ascorbic acid degradation is thought to be the major mechanism for browning in lemon juices, it was expected that the initial dissolved oxygen level of the juice would not significantly affect the browning reaction as ascorbic acid breakdown was shown to be unaffected by the three levels of dissolved oxygen. This was not the case as shown by the fact that the initial dissolved oxygen level influenced the lag period before browning became evident, the higher the initial oxygen level, the shorter the lag period. Despite the differences in lag period, the rates of browning were the same for all three oxygen levels during the post-lag period. Furthermore, although ascorbic acid degradation is the predominant source of browning, other browning reactions can take place at a temperature of 36°C. Compounds present in lemon juice such as hexose sugars may break down producing carboxylic intermediates which react to form brown polymers (Shaw et al., 1977).

Furfural and HMF formation

Furfural is an end product of ascorbic acid degradation in the pathway proposed by Bauernfeind and Pinkert (1970) and has been recommended by several workers as an index of storage temperature abuse in commercially processed citrus juices (Dinsmore and Nagy, 1972; Maraulja et al., 1973; Nagy and Randall, 1973; Kanner et al., 1982). HMF has also been proposed as an index of quality deterioration (Meydav and Berk, 1978).

The data for furfural formation during storage was best described by a zero-order reaction (Table 1). The ANACOVA showed that the different initial dissolved oxygen levels did not significantly affect furfural production. Ascorbic acid degrades anaerobically to form furfural and the presence of oxygen leads to hydroxyfurfural as well as furfural formation. These results suggest that since furfural production was not significantly affected by initial dissolved oxygen levels, the anaerobic rather than the aerobic pathway is the major one for furfural formation from ascorbic acid in lemon juice. Furthermore, as furfural can also be formed from hexose sugars (Burton et al., 1963), the role of dissolved oxygen would seem to be relatively unimportant with respect to furfural formation.

HMF formation was best described by a first-order reaction (Table 2). Meffert (1964) deduced that the initial phase of HMF formation in apple juice concentrate could be described as a half order reaction, although he quoted McGibbons (1958) as stating that the initial reaction step follows a first-order reaction mechanism. ANACOVA showed that the highest dissolved oxygen level of 3.74 mg/L resulted in significantly higher HMF production. This result is difficult to explain since the source of HMF in lemon juice would presumably be hexose sugars and oxygen plays no role in the degradative pathway.

Also worthy of note is the high initial concentration (1.31 mg/L) of HMF at zero time. At that stage the only heat treatment the juice had received was pasteurization and it is difficult to accept that this would have caused so much HMF formation. Clegg and Morton (1965) detected no HMF (determined as

their 2,4-dinitrophenylhydrazine derivatives on thin-layer chromatograms) in commercial lemon juice until after 12 days incubation at 37°C, although furfural was detected after 2 days. In the lemon juice used in the present study, the initial concentration of furfural was 0.09 mg/L. No explanation can be advanced for these high initial concentrations.

Regression analysis

Regression analysis was performed to determine the extent of the correlations between the deteriorative quality reactions taking place during storage; the correlation coefficients are presented in Table 3. For a given relationship, the correlation coefficients were very similar at the three different initial dissolved oxygen levels.

Highly significant ($P < 0.01$) correlation coefficients were obtained for the relationships between ascorbic acid degradation and furfural formation, browning index and HMF formation, browning index and furfural formation, and HMF and furfural formation. Of course, it does not necessarily follow that the relationships are adequate for accurate prediction purposes. An estimation of their usefulness in this respect can be obtained from the square of the correlation coefficient. For example, at an initial dissolved oxygen level of 0.41 mg/L, 74.6% of the variations in ascorbic acid values can be explained in terms of furfural formation, while 25.3% of the variations are not associated with furfural formation but with other factors, or with errors.

Unlike the relationships involving ascorbic acid degradation, those between browning index, HMF and furfural formation are, as well as being highly significant ($P < 0.01$), very useful for prediction purposes since the corresponding R^2 values are around 95%. These results confirm the suitability of all three parameters as chemical indices of quality deterioration in lemon juice during storage. Clegg (1964), in work on model systems, concluded that although furfural is produced during the development of browning, it did not appear to be an active participant in the formation of brown pigments. The highly significant correlations in Table 3 between browning index and furfural formation would tend to suggest a role for furfural in brown pigment formation. Certainly its formation is now well accepted as an index of storage temperature abuse in commercially processed citrus juices (Nagy and Randall, 1973; Kanner et al., 1982).

Burton et al. (1963) considered that the role of HMF in rapidly producing browning had been over-rated although they conceded that in some fruit juices, HMF may perhaps play a larger part in the overall browning. The present results illustrate that HMF concentration does have a high correlation with the level of browning in lemon juice and many therefore play an important role in the formation of brown pigments.

Table 3—Correlation coefficients for ascorbic acid retention, browning index (BI), furfural and hydroxymethylfurfural (HMF) production at three initial dissolved oxygen levels[a]

Dissolved oxygen level	BI	HMF	Furfural
0.41 mg/L			
AA	0.765	0.804	0.864
BI		0.966	0.966
HMF			0.979
1.44 mg/L			
AA	0.812	0.796	0.871
BI		0.971	0.963
HMF			0.985
3.74 mg/L			
AA	0.825	0.779	0.851
BI		0.979	0.987
HMF			0.981

[a] For 6 df, $r = 0.707$ for $P < 0.05$ and 0.834 for $P < 0.01$.

CONCLUSIONS

NO SIGNIFICANT EFFECTS on the rate of ascorbic acid degradation and furfural formation could be attributed to the different initial dissolved oxygen levels. Degradation of ascorbic acid would appear to be predominantly anaerobic and can be best described by a second-order model where the rate is proportional to the square of the ascorbic acid concentration. The lag period before browning increased depended on the initial dissolved oxygen concentration, being greater for lower initial concentrations.

Highly significant correlation coefficients were obtained between browning index, HMF and furfural formation, suggesting that all three would be suitable as chemical indices of storage temperature abuse in lemon juices.

Under commercial operations, the levels of dissolved oxygen would be sufficiently low as to make oxygen a noncritical factor in the quality deterioration of lemon juice during storage.

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Effects of Particle Size and Breadmaking on Physiological Responses of Meal-Fed Rats to AACC Wheat Bran

A-M. CADDEN

ABSTRACT

Adult rats were meal-fed diets containing 20% AACC certified hard red spring wheat bran (as received, ground, incorporated into bread) for 12 days to determine the short-term processing-induced effects on gastrointestinal function and serum lipids. Fecal nitrogen, fecal fat, transit time and serum triglyceride levels were influenced by the consumption of wheat bran, but not by the grinding or breadmaking procedures. Reduction of bran particle size decreased dietary hemicellulose and reduced fecal bulk as well as defecation frequency. Incorporating bran into bread reduced dietary neutral detergent fiber (NDF), reduced intestinal passage rate of the ground wheat bran and decreased degradation of dietary fiber components in the unground wheat bran.

INTRODUCTION

HIGH FIBER DIETS containing liberal portions of whole grain cereals, legumes, fruits and vegetables are now being advised in the treatment and management of various colonic and metabolic disorders (Staub et al., 1983). However, conventional food processing procedures such as cooking or reduction of particle size can modify physiological properties generally attributed to plant fiber. Wheat bran, the traditional source of dietary fiber added to baked goods (Dubois, 1978), is known to exert an effect on the human gastrointestinal tract (Kelsay, 1978). However, gastrointestinal function can be affected by the particle size of the wheat bran (Cadden et al., 1983; Sosulski and Cadden, 1982; Van Dokkum et al., 1983). Wyman et al. (1976) reported that cooked wheat bran had less effect on the intestine of healthy men than did a comparable amount of raw bran. Mongeau and Brassard (1984) found that rats fed puffed wheat breakfast cereal had lower fecal weights and volumes, higher fecal densities and longer transit times than rats fed either shredded wheat breakfast cereal or unprocessed wheat bran. Bjorck et al. (1984) observed that extrusion cooking allowed wheat flour to be extensively degraded in the rat intestine, but degradation of whole wheat flour was little affected by the extrusion process. Other studies have shown that water-holding capacity of wheat bran is adversely affected by particle size (Mongeau and Brassard, 1982), but not by heat (Rasper, 1979). Food processes which alter the chemical or physical state of a fiber can be expected to modify the extent of bacterial fermentation in the large intestine (Bjorck et al., 1984). This could, in turn, affect the stool bulking properties of that fiber source (Hill, 1983). More information is required concerning the effects of processing on physiologically important characteristics of dietary fiber. In this study, the possible effects of particle size and the breadmaking process on the composition and physiological attributes of AACC certified hard red spring wheat bran are examined.

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MATERIALS & METHODS

Experimental design

Sixty-four adult male Sprague-Dawley rats (total of eight rats per treatment) weighing approximately 325g were randomly allotted to each test diet, then individually housed in wire-bottomed cages equipped with individual fecal collection trays in an environmentally controlled room with 12 hr light and 12 dark (7:00 PM–7:00 AM). The collection procedures used minimized contamination of fecal matter with spilled feed and urine. Water was offered *ad libitum*; however, daily feed consumption was controlled by allowing the rats free access to their food for 2 hr (7:00–9:00 PM). The effect of the 2-hr meal feeding regime was to force the rats to consume their daily food requirements at one known time rather than throughout the day as in *ad libitum* feeding. An evening meal was chosen to coincide with the rat's nocturnal behavior patterns. The rats soon learned to consume their feed as soon as it was presented each evening, and feed consumption patterns stabilized after 4–6 days. The experimental design included a 7-day adaptation period, a 48 hr fecal collection period, and a 72 hr transit time study.

Feed consumption and excretion characteristics were assessed during the 48 hr total collection period. Feces were collected from each rat, dried at 40°C in a forced draft oven to constant weight and stored under refrigeration for chemical analysis. Fecal material dried according to the AOAC (1980) procedure contained 5.9% moisture. The reported values for dry fecal weight were corrected for this moisture content.

At the commencement of the 72 hr transit time study, the rats were given approximately 350 μg dysprosium (Kennelly et al., 1980), concentrated in 2g of test diet at the beginning of their usual evening meal (Gohl and Gohl, 1977). Test diets were also treated with 20 mg brilliant blue dye (Hess and Fitzhugh, 1955) to allow the transit time study to be followed qualitatively. After consuming the treated diet, the rats were permitted to consume their regular diet for the duration of the meal period. Stools from each rat were collected every hour for 72 hr and stored in sealed plastic bags. Each collection was weighed, dried as previously described, then reweighed. Moisture content of the stools were considered to be the difference in weight between the wet and dry stools. The quantity of dysprosium recovered at each collection was determined by neutron activation analysis (Kennelly et al., 1980). Transit time was defined as the time required for dysprosium to be first detected in the stools. Rate of passage was measured by the amount of dysprosium recovered over time.

At the conclusion of the experiment, rats were anesthetized with chloroform. Blood was collected by a heart puncture technique and, after clotting, was centrifuged at 2500 rpm (750 $\times$ g) for 15 min at 4°C. Serum was promptly removed from the separated cells with a Pasteur pipette and stored tightly capped and frozen. Serum cholesterol was measured by the enzymatic procedure of Roschlau et al. (1974) and serum triglycerides were measured by a colorimetric procedure using a kit provided by Sigma Chemical Company (No. 405).

Preparation of wheat bran

AACC certified hard red spring wheat bran (American Association of Cereal Chemists, 3340 Pilot Knob, St. Paul, MN USA 55121), was used both without further processing (WB) and after grinding in a Wiley Mill through a 1 mm screen (GWB). Unlike other studies (Sosulski and Cadden, 1982), no wheat bran particles were discarded during the preparation of GWB. Therefore, WB and GWB differed only in particle size and not in product composition.

Table 1—Percentage composition of experimental diets, dry basis

	Semipurified diets			White bread diets			Bran bread diets	
	Zero fiber	WB[a]	GWB[b]	Low fiber	WB	GWB	WB	GWB
Sucrose	61.7	45.2	45.2	--	--	--	--	--
Corn starch	6.3	6.3	6.3	6.3	6.3	6.3	6.3	6.3
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Casein	22.0	18.5	18.5	8.4	8.4	8.4	8.4	8.4
DL Methionine	0.3	0.3	0.3	0.6	0.6	0.6	0.6	0.6
AIN Mineral Mixture 76[c]	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
AIN Vitamin Mixture 76[c]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2	0.2	0.2	2.0	2.0	2.0
WB[a]	--	20.0	--	--	20.0	--	--	--
GWB[b]	--	--	20.0	--	--	20.0	--	--
White bread cubes[d]	--	--	--	75.0	55.0	55.0	--	--
WB bread cubes[d]	--	--	--	--	--	--	75.0	--
GWB bread cubes[d]	--	--	--	--	--	--	--	75.0
Water	22	26	24	120	160	150	125	125
Water:bread/bran	--	--	--	1.6:1	2.1:1	2.0:1	1.7:1	1.7:1

[a] WB — AACC(American Association of Cereal Chemists) certified hard red spring wheat bran.
[b] GWB — WB ground in a Wiley mill through a 1 mm screen.
[c] American Institute of Nutrition — based on NAS-NRC (1972) requirements for rats.
[d] Composition of breads (% on flour): unbleached flour 100.0 (14.0% moisture basis), sodium-stearoyl-2-lactylate (SSL) 0.5, vital wheat gluten 10.0, yeast 1.25, salt 2.5, sugar 8.0, nonfat dry milk 3.0, malt 0.3, ammonium phosphate monobasic 0.1, potassium bromate 75 ppm. WB bread and GWB bread also contained 45.8% WB and GWB, respectively.

Table 2—Effects of particle size and breadmaking on apparent dietary fiber composition

			Bread crumb			White bread plus wheat bran[c]	
	WB[a]	GWB[b]	White	WB	GWB	WB	GWB
Neutral detergent fiber, %	51.2	49.1	1.5	14.4	13.4	14.8	14.2
Hemicellulose, %	36.3	34.4	1.1	10.6	9.7	10.5	10.0
Cellulose, %	10.5	10.1	0.3	2.6	2.5	3.0	2.9
Acid detergent lignin, %	4.4	4.6	0.1	1.2	1.2	1.3	1.3

[a] WB — AACC (American Association of Cereal Chemists) certified hard red spring wheat bran.
[b] GWB — WB ground in a Wiley mill through a 1 mm screen.
[c] Calculated from raw material analysis (73.3% white bread crumb, 26.7% wheat bran).

Preparation of breads and diets

Experimental diets (Table 1) were based on the American Institute of Nutrition's semipurified diet for rats and mice. With the exception of the zero fiber and low fiber diets, each diet contained 20% wheat bran (as received or ground). The semipurified diets were traditional rat diets containing casein, cornstarch, sucrose and corn oil. The protein, carbohydrate and fat components were largely replaced by 55% white bread and 20% raw wheat bran (WB or GWB) in the white bread plus bran diets. In the case of the bran bread diets, the equivalent amount of WB and GWB was incorporated directly into the bread. Protein and fat not provided by WB, GWB or bread were supplied by casein and corn oil, respectively.

All breads were prepared by a 70/30 sponge dough procedure. The basic bread formula (Table 1) was adapted from Cadden et al. (1983) and Dubois (1978). The sponge containing 70% of the unbleached flour, sodium-stearoyl-2-lactylate (SSL), 40% of the vital wheat gluten, yeast, malt, ammonium phosphate and water were mixed in a large Hobart mixer until moistened (1 min) and allowed to ferment 3.5 hr at 30°C and 95% RH. Remaining ingredients, including WB and GWB, were added to the sponge and mixed in the Hobart mixer for 5 min. The dough was punched and panned by hand, using a rolling pin and baking sheet (AACC, 1982), then proofed for an additional 1.5 hr. All loaves were baked for 20 min at 230°C in a calibrated Kenmore Mark 2 household oven. After the loaves had cooled, crusts from each bread were removed and the crumb was diced into 1 cm cubes, air-dried on large fiberglass trays and refrigerated until used. Moisture content of the air-dried bread cubes was determined by standard methods (AOAC, 1980).

Bread diets were prepared by first premixing the cornstarch, casein, DL-methionine, AIN (American Institute of Nutrition) mineral mixture, AIN vitamin mixture and choline bitartrate for 20 min at low speed in a small Hobart mixer. Then, air-dried bread cubes and bran were soaked in distilled water for 20 min at room temperature (22–24°C). While the bread cubes were soaking, oil was added to the premix and mixed for a further 5 min. The soaked bread crumbs and bran were added at one time to the premix-corn oil mixture and mixed for 1 min at the lowest setting. The resulting "dough" was turned into a glass bowl and covered with plastic wrap to prevent dehydration. Each batch was quartered and the quarters from each batch were mixed together manually. Approximately 20g were removed from each quartered batch to be further treated with dysprosium and brilliant blue. The diet mass was extruded through a household cookie press into long strips approximately 0.5 cm in diameter and marked at 1 cm intervals. The extruded diets were dried in a forced draft oven for 24 hr at 40°C, broken at appropriate lengths and stored in sealed plastic bags in the refrigerator.

In the case of the semipurified diets, a premix containing cornstarch, casein, DL-methionine, AIN mineral mixture, AIN vitamin mixture and choline bitartrate was prepared and thoroughly mixed for 20 min at low speed in the small Hobart mixer. Corn oil was added to the premix and mixed for an additional 5 min. Sugar was dissolved in boiling water and brought to a rolling boil for 1 min. The syrup was added to the premix-corn oil mixture and mixed until pasty white (2 min). Wheat bran was folded into the mixture and mixed for a further 1 min. The purpose of this extensive diet preparation procedure was twofold: (1) to avoid confounding the effect of particle size by breaking the WB and GWB contained in the bran breads, and (2) to increase the homogeneity of the diets by removing the possibility of the animals being able to select or avoid certain dietary components.

Analytical procedures

Dietary fiber composition of WB and GWB, bread cubes, diets and feces were determined by the sequential detergent fractionation scheme recommended by Robertson and Van Soest (1981). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined by the procedures of Goering and Van Soest (1970). The neutral detergent procedure included an 18 hr incubation treatment with alpha-amylase from hog pancreas (Sigma #A6880) as outlined by AACC (1982). ADF and ADL determinations were carried out on residues recovered during NDF analyses. Hemicellulose was calculated as the difference between NDF and ADF. Cellulose was estimated by the amount of ADF residue dissolved in 72% sulfuric acid during the ADL analysis. To avoid confounding data related to particle size, the samples were allowed to soak in the test reagent at room temperature for 1 hr prior to refluxing. This allowed the samples to imbibe the reagent and disintegrate into suitably small particles prior to analysis. Results using this modified procedure were consistent with raw material analysis.

Diets and fecal samples, uncontaminated by spilled feed and urine,

were analyzed for nitrogen by the Kjeldahl method and for fat by acid hydrolysis using standard procedures (AOAC, 1980).

Statistical procedures

The effect of wheat bran supplementation, the effect of the diet base (semipurified, white bread or bran bread), and the effect of the reduction in wheat bran particle size were assessed using analysis of variance and orthogonal coefficients. Means were compared by Duncan's Multiple Range Test.

RESULTS & DISCUSSION

THE RAW WB contained slightly higher levels of NDF and insoluble hemicellulose than raw GWB (Table 2), but cellulose and ADL levels were similar. Breads containing GWB contained less NDF and hemicellulose than WB-breads. This suggested that the insoluble hemicellulose fraction of wheat bran was reduced by the grinding procedure.

The effect of the breadmaking process on apparent dietary fiber composition was examined by comparing the composition of the bran breads with values calculated for mixtures of white bread and raw wheat bran (Table 2). NDF and cellulose values for bran breads were lower than expected from raw material analysis, the reduction of NDF being greater in the case of GWB than WB. The lower NDF and cellulose values for bran breads suggested that the hydration/heat/fermentation processes of breadmaking may have hydrolyzed certain polysaccharide bonds effectively decreasing the level of insoluble dietary fiber (NDF).

Omaye and Chow (1984) reported that meal-fed rats consumed less food, gained less body weight, had larger intestinal tract weight and lower plasma fat-soluble vitamin levels than nibbling rats fed the same diets. Therefore, some caution should be exercised in comparing results obtained with meal-fed and *ad libitum* fed rats due to differences in their physiology and metabolism.

All rats consumed similar amounts of feed during the test period, irrespective of diet (Table 3). Body weights were maintained. Inclusion of wheat bran in the rat diet increased fecal weight and defecation frequency, as well as fecal excretion of nitrogen and fat. Reduction of bran particle size reduced the stool bulking effect of the wheat bran as measured by fecal wet weight and defecation frequency. Other investigators (Cummings, 1978; Kelsay, 1978; Van Dokkum et al., 1983) have reported similar results with human subjects. Incorporating wheat bran into bread had no effect on the weight of fecal stools excreted by the rats; however, defecation frequency was lower when rats were fed the corresponding mixture of white bread and raw wheat bran. Neither process (grinding, breadmaking) affected nitrogen and fat excretion.

Wheat bran had no effect on the serum cholesterol levels of the rats (Table 3). This was consistent with the work of Forsythe et al., 1978 and Kelsay (1978). As reported in the study by Munoz et al., 1979, serum triglyceride levels were reduced by the inclusion of wheat bran in the diet, perhaps due, in part, to the increased fat excretion. Neither process (grinding, breadmaking) affected serum triglyceride levels.

Inclusion of wheat bran in the diet reduced the time required for the dysprosium marker to appear in the feces (Table 3). Particle size had no effect on transit time of the bran-fed rats; however, incorporating GWB into bread increased the time required for the initial appearance of the dysprosium marker. Similar results were obtained by recording the first appearance of the brilliant blue dye.

Dysprosium continued to be excreted for a substantial time period, following its initial appearance (Fig. 1, 2), the retention of digesta being dependent upon the diet as well as the individual animals. This was consistent with the observations of other workers (Lutwak and Burton, 1964) using dyes as fecal markers. The shape of the dysprosium excretion curve differed between bran-fed rats and rats fed the zero fiber and low fiber diets. The flatter slope and low recoveries associated with the non-bran diets illustrates the slower passage rate of these diets. The time required to recover 50% of the dysprosium dose (Table 3) was consistent with transit time data. That is, the inclusion of wheat bran in the diet reduced the amount of time required for digesta to travel through the gastrointestinal tract of the rat, but processing (grinding, breadmaking) of the bran had no significant effect ($P = 0.05$). As in the study by Luckey et al. (1975), most of the dysprosium dose administered to the bran-fed rats, was excreted after 24 hr ($P = 0.05$). However, rats fed GWB-bread excreted less dysprosium than rats fed other bran diets during the initial 24 hr period ($P = 0.05$), indicating that the breadmaking process combined with the reduction of wheat bran particle size decreased the passage rate of digesta through the gastrointestinal tract.

Fecal excretion of NDF, hemicellulose, cellulose and lignin increased with the addition of wheat bran to the rat diet (Table 4). Differences concerning the disappearance and recovery of dietary fiber components were related to the action of colonic bacteria in the gastrointestinal tract of the rat (Nyman and Asp, 1982). Wheat bran is known to be relatively resistant to microbial fermentation. Particle size of the wheat bran did not have a significant effect ($P = 0.05$) on recovery of dietary fiber constituents; however, rats fed the WB-bran bread excreted

Table 3—Effects of particle size and breadmaking on physiological parameters[a]

	Semipurified diets			White bread diets			Bran bread diets		Standard
	Zero fiber	WB	GWB	Low fiber	WB	GWB	WB	GWB	error
Feed intake, g/day	14a	13a	13ab	12ab	11b	11b	12ab	12ab	0.5
Excretion characteristics									
Fecal weight, g/day									
— wet	0.8d	3.9a	3.5ab	1.3c	3.6ab	3.3b	3.7ab	3.4ab	0.2
— dry	0.3a	1.6c	1.6c	0.5b	1.6c	1.5c	1.7c	1.5c	0.1
Defecation frequency, stools/hr	0.4d	1.0a	0.9a	0.5d	1.0a	0.9b	0.9b	0.8c	0.1
Fecal nitrogen									
— mg/day	24c	62c	54a	39b	52a	57a	59a	60a	3
— % of intake	8d	19a	15b	13c	18a	19a	19a	18a	1
Fecal fat									
— mg/day	71c	109a	99a	76a	103a	96ab	96ab	99a	7
— % of intake	15c	23a	22ab	19b	20ab	19b	18b	19b	1
Serum lipids, mg/100 ml									
Cholesterol	82a	105a	97a	110a	94a	88a	80a	138a	24
Triglycerides	118a	62b	56b	97a	59b	77b	38b	44b	18
Transit time and rate of passage									
First appearance of Dy, hr	23a	9cd	10bc	12bc	8d	8d	11bcd	13b	1
50% recovery, hr	47a	16d	19cd	33b	18cd	19cd	21cd	24c	3
Dy recovery after 24 hr, %	1e	77a	64ab	19b	76ab	69ab	63b	41c	6

[a] Means within the same row followed by the same letter are not significantly different ($P = 0.05$).

Table 4—Degradation of dietary fiber components[a]

	Semipurified diets			White bread diets			Bran bread diets		Standard
	Zero fiber	WB	GWB	Low fiber	WB	GWB	WB	GWB	error
NDF, mg/day									
— consumed	0d	107a	110a	10d	92b	79c	94b	89bc	4
— excreted	4d	106a	93ab	9d	75c	82bc	98a	94ab	5
						36bc	39ab	38ab	2
Hemicellulose, mg/day									
—consumed	0d	77a	77a	7d	65b	56c	66b	66b	3
—excreted	0c	28a	27a	2c	21b	23ab	26a	26a	1
Cellulose, mg/day									
—consumed	0f	21b	24a	2e	18c	13d	19bc	15d	1
—excreted	0c	26a	25a	2c	20b	22ab	24a	24a	1
ADL, mg/day									
—consumed	0c	9ab	9ab	0c	9ab	10a	9ab	8b	1
—excreted	2d	15a	13b	2d	10c	12b	13b	12b	1

[a] Means within the same row followed by the same letter are not significantly different (P = 0.05).

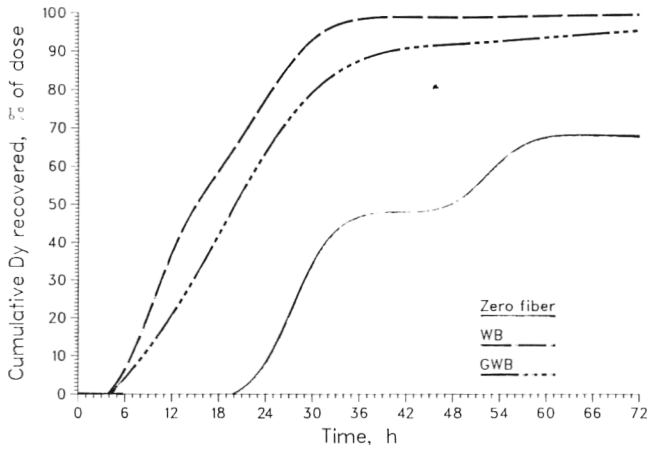

Fig 1—Dysprosium (Dy) recovery over 72 hr for meal-fed adult rats fed semipurified diets. Each curve represents the mean recovery from eight rats.

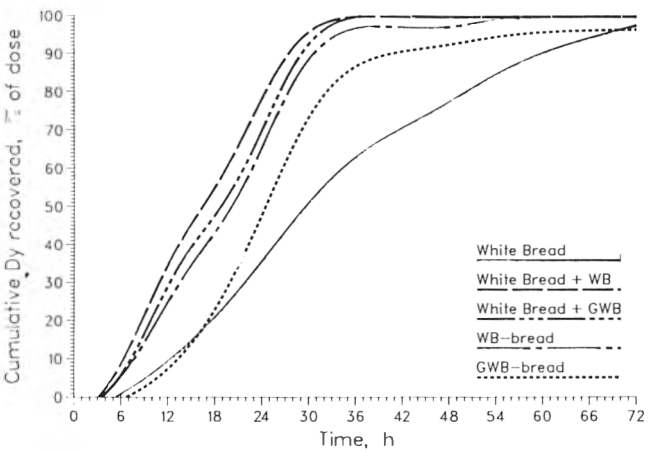

Fig 2—Dysprosium (Dy) recovery over 72 hr for meal-fed adult rats fed bread diets. Each curve represents the mean recovery from eight rats.

more NDF, hemicellulose, cellulose and ADL than rats fed the corresponding white bread/raw WB diet. This effect was not significant (P = 0.05) in the case of the GWB-bread diets. Perhaps degradation of dietary fiber constituents in the bran bread diets was impaired by the presence of Maillard reaction products produced in the baking process (Spiller and Amen, 1975). The lesser effect of heat on GWB could be attributed, in part, to the slower passage rate of the GWB-bread diet, and, in part, to the lesser amount of insoluble dietary fiber present in the GWB-bread. A more severe heat treatment may have induced larger effects on the physiological parameters measured.

The importance of cereal fiber in the diet has been recognized by the medical profession, especially in the treatment and management of certain colonic and metabolic disorders. One physiological characteristic that has been found to be closely associated with the prevalence of these conditions is the size and consistency of fecal stools (Burkitt et al., 1972). Large soft stools have been associated with low prevalence of these disorders, whereas high prevalence has been associated with small firm stools. The desirable stool bulking properties of wheat bran can be diminished by reducing bran particle size. Incorporating wheat bran into baked goods, such as bread, will probably not impair the stool bulking properties of the bran; however, other physiological parameters (rate of digesta passage, degradation of dietary fiber components) may be affected.

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ELISA DETECTION OF MOLD IN TOMATO PUREE. . .From page 183

ELISA procedures such as just described have considerable potential for replacing existing methods for the detection of molds in food products. ELISA is less subjective than the HMC and, as carried out here, was more sensitive for detecting mold in tomato puree than the previously described HPLC method (Lin and Cousin, 1985), in which the breakdown product of fungal chitin was measured and the detection limit was approximately 0.1 mg dried mold/g of sample.

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Emulsifying Properties of Ethanol Soaked Soybean Flour

HEA-RAN LEE ASHRAF

ABSTRACT

Soy flours prepared from soybeans soaked at 60°C for 6 hr in distilled water, 15% ethanol, or 0.1M $NaHCO_3$ in 15% ethanol were tested for their emulsifying capacity (EC) at pH 4.5, 6.5, and 9.0. At pH 4.5 and 6.5, there were no significant differences in EC among treatments; at pH 9.0, the EC was highest for soy flour from beans soaked in water, intermediate for flour from beans soaked in 15% ethanol, and lowest for flour from beans soaked in 0.1M $NaHCO_3$ in 15% ethanol. The relationship between the amount of soluble protein and EC was inverse with EC decreasing sharply up to about 60 mg soluble protein/50 mL of flour dispersion. Photomicrographs of air incorporation during emulsion formation are presented.

INTRODUCTION

THE USE of soy protein products in a wide variety of food products is expanding rapidly. Such applications require proteins with superior functional properties. Heating has been the most successful process for reducing undesirable activities, such as lipoxygenase activity, in soybeans; but decreased functional properties could be a major disadvantage of heating (Kinsella, 1979). The effect of heating on emulsification properties of soy and other plant proteins has been determined (Aoki et al., 1980; McWatters and Holmes, 1979a, b; Hutton and Campbell, 1977).

Alcohol soaking or washing of soybeans and soybean products is effective in removing off-flavors (Eldridge et al., 1977; Rackis et al., 1979). Heating and the addition of sodium salts decrease the ethanol soaking period required and increase nitrogen solubility compared to heating alone (Borhan and Snyder, 1979). Ashraf and Snyder (1981) observed that soaking soybeans at 60°C in 15% ethanol containing sodium carbonate and sodium bicarbonate, not only eliminated the grassy, beany flavor, but also intensified the nutty flavor of soy milk.

The present study investigated the effect of this treatment on the emulsification properties of soybean flour.

MATERIALS & METHODS

Soybeans

The Williams variety (seed bean quality) with proximate composition of 8.1% moisture, 36.4% protein, 19.7% crude lipid, 5.0% ash and 30.8% carbohydrate (by difference) was used.

Soybean oil

A commercially available partially hydrogenated soybean oil with a fatty acid composition of $C_{16:0}$, 10.8%; $C_{18:0}$, 4.2%; $C_{18:1}$, 38.7%; $C_{18:2}$, 41.2%, and $C_{18:3}$, 4.4% (Kroger CO., Cincinnati, OH) was used.

Soaking

Soybeans (100g) were soaked for 6 hr at 60°C in 300 mL of (1) distilled water, (2) 15% ethanol, and (3) 0.1M $NaHCO_3$ in 15% ethanol solution. Distilled water soaking at room temperature (23°C) was also done to evaluate the effect of soaking without heating. The dehulled beans were resoaked overnight in distilled water at 4°C to eliminate residual chemicals.

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Flour preparation

Treated beans were freeze-dried in a Thermovac freeze-drier and pulverized using a Wiley mill with 80 mesh screen. Lipids were extracted from the flour with petroleum ether.

Proximate analysis

Proximate analysis for soybean and flour samples was determined by standard AOAC procedures (AOAC, 1970) viz., vacuum oven for moisture, micro-Kjeldahl for protein, ether extraction for crude fat, and the muffle furnace method for ash. Carbohydrate was calculated by difference. All analyses were done in triplicate. The results are given in Table 1.

Protein solubility

Dispersions of 1g of flour in 20 mL of distilled water were adjusted to pH 4.5, 6.5, and 9.0 with 0.1N HCl or 0.1N NaOH. After adjusting the volume of the dispersion to 25 mL, it was stirred for 1 hr and then centrifuged at 10,000 rpm for 15 min. A 5 mL aliquot of pooled supernatant was analyzed for protein by the micro-Kjeldahl method (AOAC, 1970).

Emulsification capacity

Emulsions were formed from 50 mL of 1% soy flour suspensions in distilled water at pH 4.5, 6.5, and 9.0 using an Osterizer blender at approximately 6,000 rpm. Partially hydrogenated soybean oil was added from a burette through the top opening of the blender jar cover at an average rate of 15 mL per min. This method is a slight modification of the methods used by Ramanatham et al. (1978) and McWatters and Holmes (1979a, b). The breaking point was determined by a sudden drop of viscosity and a concurrently distinctly visible separation of the two phases. Emulsification Capacity (EC) was expressed as ml of oil per mg of protein. EC was measured in triplicate. Analysis of variance and the least significant difference test (Steel and Torrie, 1960) were applied to determine the effects of the soaking conditions.

Light microscopy

Samples of emulsions at different oil levels were obtained at various stages of mixing, mounted on slides and examined by phase contrast microscopy at 213× magnification. Emulsions were prepared at a concentration of 0.5 g/50 mL distilled water.

RESULTS & DISCUSSION

Effect of heating at 60°C

Generally, heat treatment reduces the Emulsification Capacity (EC) of proteins. Figure 1 shows the EC of soy flours prepared from beans soaked in water at room temperature (23°C) and 60°C. Samples soaked at 60°C retained 35%, 86%, and 75% of the EC of unheated samples at pH 4.5, 6.5, and 9.0, respectively.

In a study on emulsifying properties of soy proteins, Aoki et al. (1980) indicated that the EC of the 7S and 11S protein-rich fractions (PRF) were decreased as a result of heating at temperatures between 50°C and 95°C, with the lowest value being obtained at 85°C. The 11S PRF showed a greater decrease in the EC than did the 7S PRF. The duration of heating is the main determinant in reducing the EC of soybean proteins

Table 1—Composition of defatted flours obtained from treated soybeans

Soaking condition	Crude protein %	Lipid %	Moisture %	Ash %	Carbohydrate %
H_2O, 23°C for 6 hr	50.6	2.3	12.0	5.4	29.6
H_2O, 60°C for 6 hr	56.7	2.2	11.7	3.3	26.0
15% ETOH, 60°C for 6 hr	53.7	1.9	12.4	3.8	28.1
0.1M $NaHCO_3$ in 15% ETOH, 60°C for 6 hr	52.7	2.1	11.6	4.5	28.9

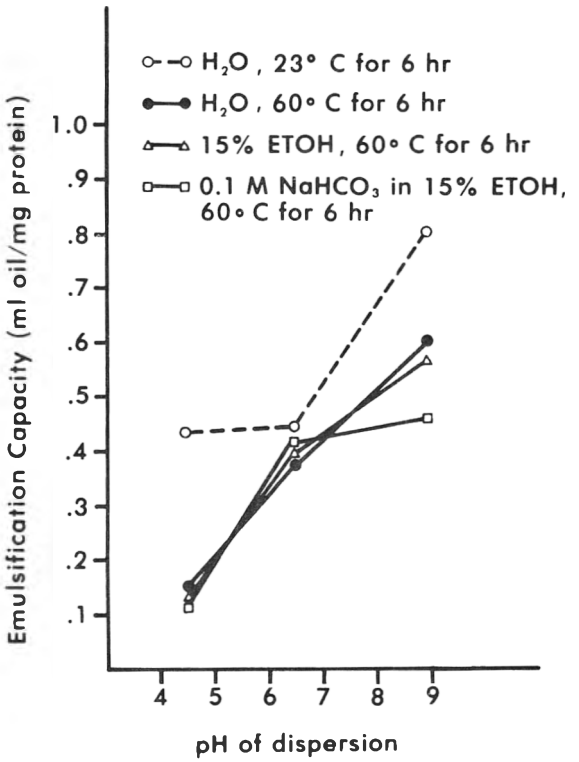

Fig. 1—Effect of soaking conditions on EC of the soybean flours.

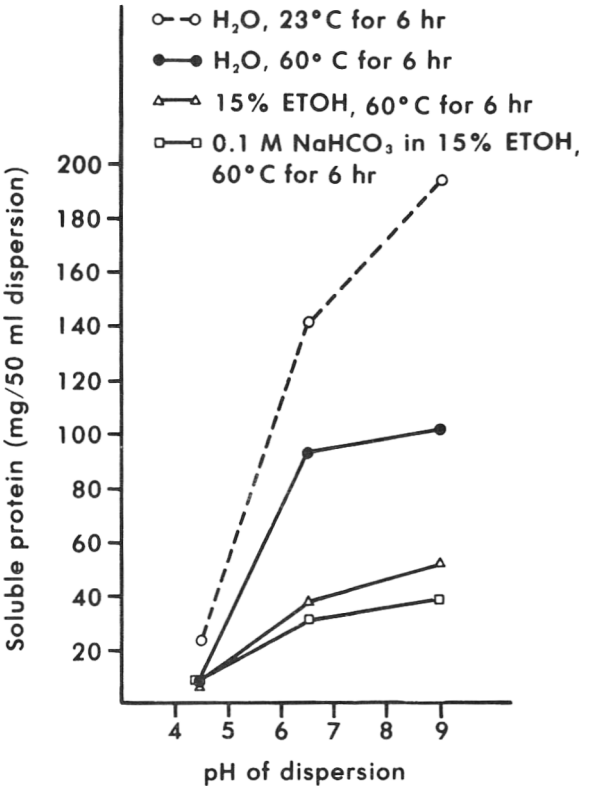

Fig. 2—Effect of soaking conditions on protein solubility of the soybean flours.

when soy flour is steam-heated at 100°C (McWatters and Holmes, 1979a).

Effect of pH

The EC of samples varied with the pH of the dispersion from which emulsions were formed (Fig. 1). At pH 4.5, the ECs of heat-treated samples were much lower than that of the unheated sample. Among the heat-treated samples, no significant difference in EC was observed at pH 4.5 or 6.5; however, the differences at pH 9.0 were significant ($p \leq 0.001$). For example, flour from beans soaked in 0.1M $NaHCO_3$ in 15% ethanol retained only 76% of the EC of flour from beans soaked in water at 60°C. For all treatments, the EC increased with increasing pH, markedly so except for the water soak at room temperature between pH 4.5 and 6.5 and the 0.1M $NaHCO_3$ in 15% ethanol soak at 60°C between pH 6.5 and 9.0. The emulsifying activity of proteins is determined by their ability to diffuse to the interface and form a film around the lipid globules. This ability of protein is affected by their conformation, spreadability, flexibility, and hydrophobicity (Phillips, 1981; Aoki et al., 1984). The initial absorption of a protein on lipid globules occurs by hydrophobic interactions between the protein and the lipid surface. The effective hydrophobicity of proteins can be altered by pH because of the pH dependency of their conformations (Yamauchi et al., 1980).

Effect of ethanol soaking

Figure 1 also shows EC of samples soaked for 6 hr at 60°C in water, in 15% ethanol, and in a solution containing 0.1M $NaHCO_3$ in 15% ethanol. From this figure, it can be seen that at pH 4.5 and 6.5 soaking in either 15% ethanol or 15% ethanol containing $NaHCO_3$ did not reduce EC as compared to soaking in water at 60°C: the differences in EC between the three treatments were not significant ($p \leq 0.01$). The same result was obtained in unpublished data using peanut oil. Considering the improvement of flavor in these samples (Ashraf and Snyder, 1981) increased EC is another advantage of the treatment. At pH 9.0, however, soaking in both 15% ethanol and 15% ethanol containing $NaHCO_3$ resulted in significantly lower EC values than did soaking in water.

In general, the denaturing power of organic solvents depends on their hydrophobicity and concentration in water. Short chain alcohols are stronger denaturants than are other organic solvents. At low concentration, the denaturing power increases with the hydrophobicity of alcohols; at high concentrations, the reverse is true. This phenomenon is explained by the fact that hydrophilic regions are oriented towards the exterior and hydrophobic regions are oriented towards the interior of a globular protein. Thus, disruption of the hydrophilic region by aqueous solutions must occur before hydrophobic region are disrupted by alcohols (Fukushima, 1969). Roberts and Briggs (1963) reported that the 7S component of soybean globulin is most sensitive to alcohol denaturation; 11S and 15S proteins are also denatured, but only slowly; and the 2S component is not denatured. It is reported that 7S and 11S soy proteins and soy protein isolate treated in ethanol (40–60%) at 25°C for 30 min had similar emulsification activity to that of the untreated protein (Hirotsuka et al., 1984).

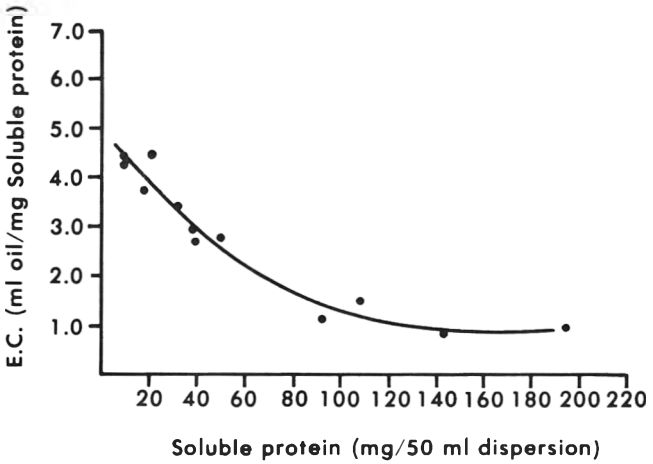

Fig. 3—Emulsification capacity as a function of soluble protein concentration of the dispersion.

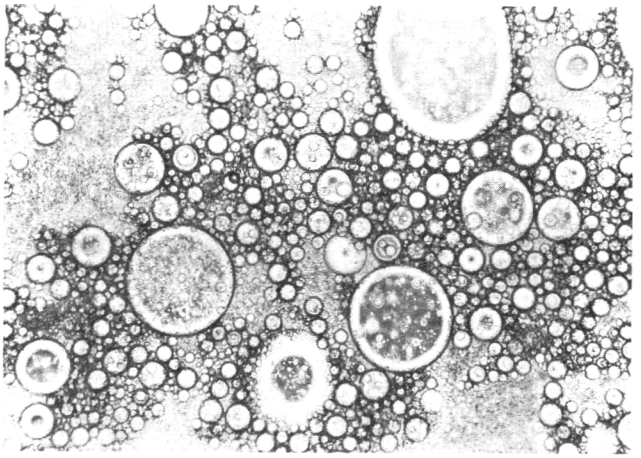

Fig. 4—Photomicrograph (213×) of an emulsion from water soaked (60°C) soy flour at 50 mL oil level per 50 mL of dispersion.

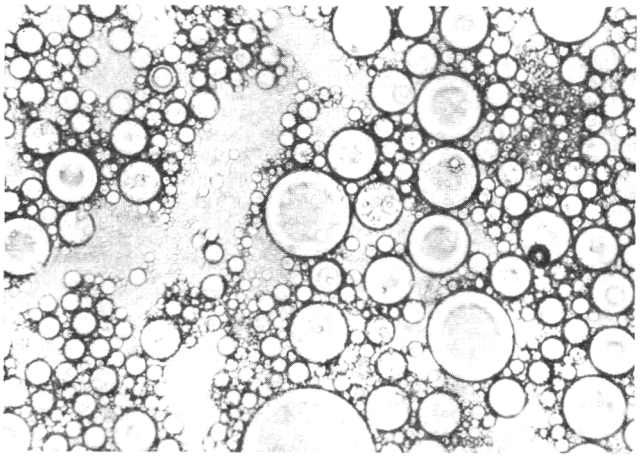

Fig. 5—Photomicrograph (213×) of an emulsion from $NaHCO_3$-ethanol soaked soy flour at 50 mL of oil per 50 mL of dispersion.

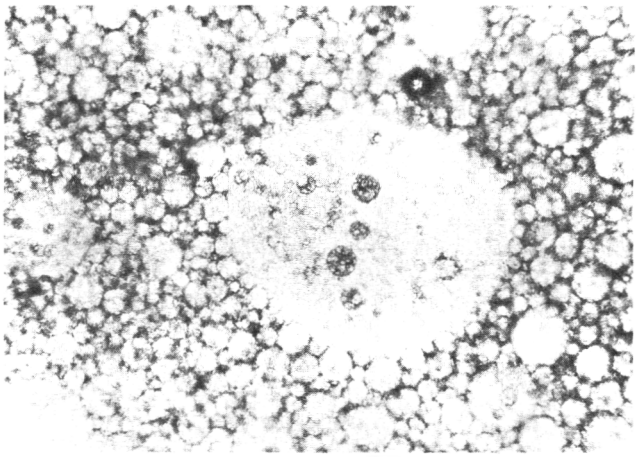

Fig. 6–Photomicrograph (213×) of an emulsion from water soaked (60°C) soy flour at 100 mL oil level per 50 mL of dispersion.

Protein solubility

EC curves of treated samples generally resembled the protein solubility profile of the same samples; that is, solubility increased with increasing pH between pH 4.5 and pH 9.0 (Fig. 2). However, the rates of increase were not identical. To examine the relationship between protein solubility and EC, EC was plotted against soluble protein in the dispersion (Fig. 3). The relationship was similar to that of EC and total protein in various types of protein emulsions (Aoki, 1981; Ramanatham et al., 1978; Pearson et al., 1965; Carpenter and Saffle, 1964). It was observed that the EC was inversely proportional to the soluble protein content in a nonlinear fashion, with the greatest change taking place up to 60 mg per 50 mL of dispersion. In other words, the emulsifying efficiency decreased rapidly with increases in the concentration of soluble protein.

Other reports indicate similar relationships (Ramanatham et al., 1978; Crenwelge et al., 1974), although some investigators have suggested that nitrogen solubility is not necessarily associated with EC of vegetable proteins (Aoki et al., 1980; McWatters and Holmes, 1979a,; Wang and Kinsella, 1976).

Microscopic examination

Emulsions were prepared for microscopic examination at pH 9.0 because of the superior emulsifying capacity of proteins at this pH. Photomicrographs of emulsions from water (60°C) and $NaHCO_3$-ethanol soaked flours are presented in this section.

At 50 mL oil level, sizes of oil droplets were irregular and the surrounding layers were thick (Fig. 4 and 5). The color of the emulsion became white and consistency was very thin. As the oil level increased to 100 mL, oil droplets became uniform in size, but consistency was thin similar to that of a pourable salad-dressing (Fig. 6 and 7). The large light spots in the middle of Fig. 6 and 7 seemed to be air bubbles trapped in the emulsion. Fig. 8 shows an emulsion of water soaked flour at 140 mL of oil. The emulsion became extremely thick, making mixing difficult. Oil droplets were still small and uniform, and more air bubbles of smaller size were incorporated in the emulsion. Air bubbles were surrounded by oil droplets, and being much larger than oil droplets, they appeared three dimensional. This could be the result of excess soluble protein which was not involved in emulsion formation but took part in air incorporation forming a product similar to dairy whipped cream. The phenomenon of water-soluble protein participating in air incorporation might influence the EC of proteins due to dual functions of the proteins. Also emulsion viscosity could be affected by air bubbles in the system. For instance, an emulsion with a large number of air bubbles with rather stiff structure did not have fluidity. Sodium bicarbonate-ethanol soaked samples did not reach this stage of air incorporation. Emulsions reached breaking points at 170 mL and 120 mL of oil levels with water-soaking and $NaHCO_3$-ethanol soaking, respectively, (Fig. 9 and 10). From these photomicrographs, it was noted that water-soaking increased both size and number of continuous membrane-like debris (Fig. 9), whereas with $NaHCO_3$-ethanol soaking, large numbers of small debris (A)

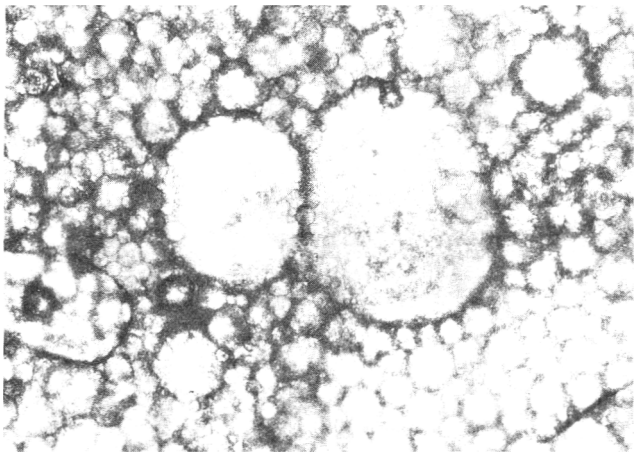

Fig. 7—Photomicrograph (213×) of an emulsion from $NaHCO_3$-ethanol soaked soy flour at 100 mL of oil per 50 mL of dispersion.

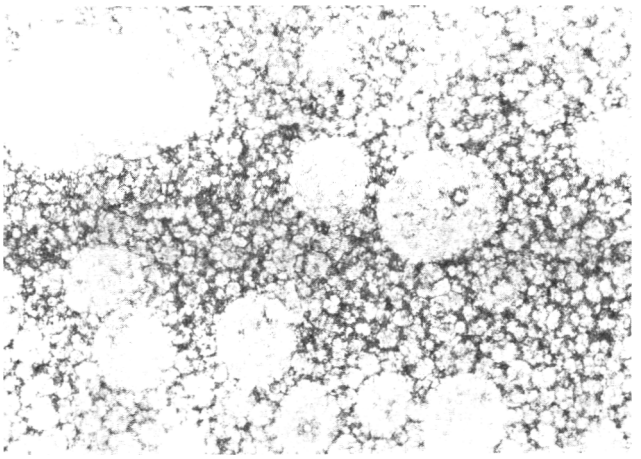

Fig. 8—Photomicrograph (213×) of an emulsion from water soaked (60°C) soy flour at 140 mL oil level per 50 mL of dispersion.

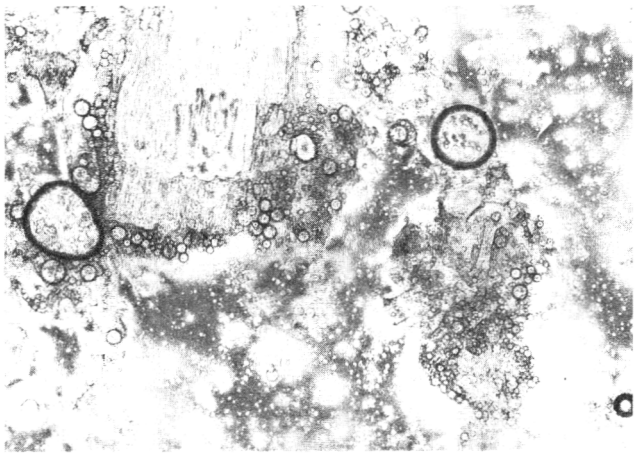

Fig. 9—Photomicrograph (213×) of a broken emulsion from water soaked soy flour.

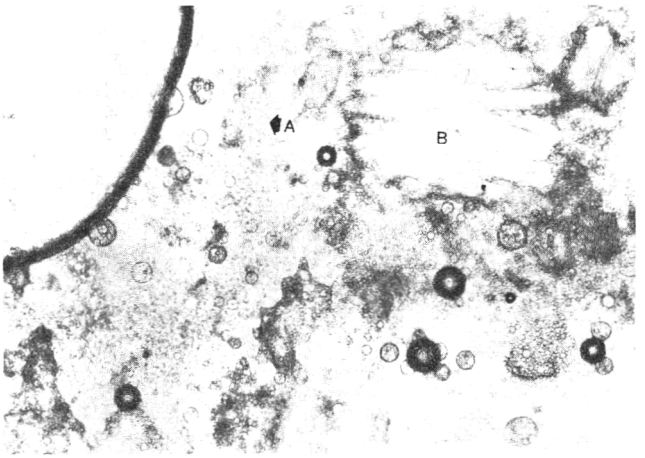

Fig. 10—Photomicrograph (213×) of a broken emulsion from $NaHCO_3$-ethanol soaked soy flour. (A) Small debris; (B) Continuous membrane-like debris.

were observed along with large debris (B) (Fig. 10). This could be due to more extensive denaturation of proteins which resulted in less flexible configurations.

Ethanol washing of soybean flakes is known to be very effective in improving flavor. The effect of ethanol on functional properties of these treated products is generally unknown. The information on emulsification should be useful since many of the food products in which soy protein products are incorporated contain emulsion systems. Ethanol treatments of soybean flours may be used for human food purposes, but further information is needed to better understand the behavior of these products in food systems and to increase the utilization of soybeans.

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Chlorine Dioxide Inactivation of *Bacillus* and *Clostridium* Spores

P. M. FOEGEDING, V. HEMSTAPAT, and F. G. GIESBRECHT

ABSTRACT

Bacillus cereus T and F4810/72, *B. stearothermophilus* ATCC 1518, and *Clostridium perfringens* NCTC 8798 spore inactivation by 20, 50, and 80 mg chlorine dioxide (ClO_2)/L at three pH values (4.5, 6.5, 8.5) were evaluated. ClO_2 concentration, species and sporulation medium significantly ($p < 0.001$) affect spore sensitivity. *B. cereus* T sporulated in Modified G medium and *B. stearothermophilus* ATCC 1518 are similarly ClO_2 sensitive, and each is significantly more sensitive than Fortified Nutrient Agar-sporulated *B. cereus* T and F4810/72. The *Bacillus* spore populations are more ClO_2 sensitive than *C. perfringens* spore populations. Treatment pH affects *C. perfringens* but not *Bacillus* inactivation. Spores lacking intact coats are significantly more ClO_2 sensitive than the strains with coats intact.

INTRODUCTION

CHLORINE COMPOUNDS (hypochlorite, hypochlorous acid, and Cl_2) are the disinfectants used most widely in water and wastewater treatment, and also are used commonly as food sanitizers. Chlorine treatment of water which contains residual organic compounds or contact with organic material may result in the formation of halogenated organic compounds (Page et al., 1976; Wei et al., 1985). Concern over the carcinogenicity and toxicity of the halogenated organic compounds, particularly trihalomethanes, has prompted consideration of alternative disinfectants. A number of investigations have indicated chlorine dioxide (ClO_2) is a disinfectant equal to or more effective than chlorine. However, ClO_2 does not result in formation of chlorinated organic compounds to the extent observed with chlorine. Chlorine dioxide currently is used in the food industry in applications such as treatment of vegetable fluming water and its potential use has been explored for other applications (Lillard, 1979).

Chlorine dioxide was broad biocidal effectiveness. ClO_2 has been reported to inactivate effectively bacteria (Ridenour and Ingols, 1947; Ridenour and Armbruster, 1949; Benarde et al., 1965), viruses (Cronier et al., 1977), bacterial spores (Ridenour et al., 1949), and algae (White, 1972). Information on the sporicidal activity of chlorine dioxide is limited. To realize broad application in the food industry, knowledge of the sporicidal effectiveness of ClO_2 is needed since many sanitation processes are designed to control bacterial spores.

One objective of this study was to assess the difference in ClO_2 sensitivity among spores of various genera, species, and strains including the effect of ClO_2 concentration and exposure pH and the influence of spore preparation procedure. A second objective was to determine the role of the spore coat in protection against ClO_2.

MATERIALS & METHODS

Test organisms

Cultures were obtained from the following sources: *Bacillus cereus* T and *B. cereus* F4810/72, from K. Johnson (Dept. of Food Science & Nutrition, Univ. of Minnesota); *B. stearothermophilus* ATCC 1518 and *Clostridium perfringens* NCTC 8798 from the North Carolina State Univ. culture collection; *C. perfringens* 8-6 spores, a coatless mutant of NCTC 8798, from L. Sacks (USDA Western Regional Research Center, Berkeley, CA).

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Preparation of spore suspensions

Sporulation. *B. cereus* T and *B. cereus* F4810/72 were sporulated using Fortified Nutrient Agar (FNA) according to the method of Johnson et al. (1982). Two populations of *B. cereus* T spores were produced on separate occasions by this procedure. *B. cereus* T also was sporulated using Modified G medium (MGM) as detailed by Bhothipaksa and Busta (1978). *B. stearothermophilus* ATCC 1518 spores were prepared as detailed by Kimsey et al. (1981).

Sporulation of *C. perfringens* NCTC 8798 and 8-6 was in D-medium using a modification of the procedure of Sacks and Thompson (1978). Methylxanthines were not incorporated into the D-medium. One loopful of a spore suspension (ca 10^7 spores/mL) was transferred into 9 mL freshly prepared Fluid Thioglycolate medium (FTG, Difco Laboratories, Detroit, MI). The spores were heat activated in the FTG for 20 min at 75°C and incubated 18–20 hr at 37°C. This FTG culture was used to inoculate 50 mL freshly steamed FTG (10% inoculum) in a 25 × 250-mm screw-cap test tube. The culture was incubated 18–20 hr at 37°C and used to inoculate a final 50 mL FTG as detailed above. After 18–20 hr incubation at 37°C, a 5% inoculum of the fresh FTG culture was used to inoculate 1L D-medium freshly prepared in a 1L volumetric flask. The inoculum was introduced deep into the D-medium and incubation was at 37°C until ≥90% of the cells had sporulated (24 hr and 84 hr for NCTC 8798 and 8-6, respectively) as determined by phase-contrast microscopy.

Harvesting, washing, and storing spores. For the spores produced on agar medium (FNA-sporulated *B. cereus* and *B. stearothermophilus*), about 10 mL sterile distilled water was added to each plate and the spores were scraped from the surface using a sterile bent glass rod and collected for centrifugation at 1000 × *g* for 20 min at 4°C. Each plate was rinsed two to three times with water to collect the majority of the spores. The spore pellet was resuspended for washing in cold sterile, deionized water. The centrifugation speed was increased by 500 rpm for each wash to a maximum of 3000 × *g*. Spore pellets were washed five times and were essentially free of vegetative cells or cell debris as determined by phase-contrast microscopy (1500 × magnification). The spore pellets were pooled and resuspended in ca 100 mL deionized water. Spore suspensions were shaken (ca. 150 rpm) overnight at 4°C with sterile glass beads (3-mm diameter) to disperse clumps of spores. Shaking was repeated until direct microscopic counts (DMC) using a Petroff-Hausser counting chamber and colony counts were essentially identical (DMC no greater than 2.5 times colony count). *Bacillus* spore suspensions were held in deionized water and stored at 2–4°C unless otherwise indicated.

B. cereus T sporulated on MGM was harvested by centrifugation (1000 × *g*, 4°C, 20 min). Spores were washed, shaken with glass beads, and stored as detailed above.

C. perfringens NCTC 8798 and 8-6 spores were collected from the D-medium by centrifugation (1000 × *g*, 1–2 hr, 4°C). *C. perfringens* NCTC 8798 spores were washed 6 times and were held in deionized water at 2–4°C. *C. perfringens* 8-6 spores were held in spent D-medium at 2–4°C since holding in deionized water was found to alter lysozyme dependence and viability.

Coat removal procedure. Coats were removed from a portion of one FNA-sporulated *B. cereus* T spore suspension using the method of Aronson and Fitz-James (1968). In this procedure, 10^9 washed spores were sedimented (1000 × *g*, 20 min, 4°C) and the pellet suspended in 2.0 mL of freshly dissolved 50 mM dithiothreitol (DTT) plus 5% sodium dodecyl sulfate (SDS) in 0.1M NaCl. The pH of the suspension was raised to pH 10.5 with 2.0N NaOH, and the suspension was incubated 18–24 hr at 37°C. The DTT plus SDS-treated *B. cereus* T spores were washed eight times and shaken with glass beads

as detailed above prior to storing at 2–4°C in 0.3M sucrose (pH 9.0) to prevent clumping of the spores (Aronson and Fitz-James, 1968).

Testing for coat integrity. Germination of spores without coats or with permeable coats can be effected by lysozyme while lysozyme-induced germination of spores with intact coats is absent or poor. To determine the percentage of DTT plus SDS-treated *B. cereus* T spores with altered or removed coats, spores (2×10^6/mL) were incubated at 35°C in 100 μg lysozyme/mL of 0.05M sodium phosphate buffer, pH 7.6. At selected times an aliquot was removed to determine the extent of germination by enumerating heated (70°C, 15 min) and unheated samples using Trypticase Soy Agar (TSA, Difco Laboratories, Detroit, MI). Results indicated 100% germination (100% heat sensitivity) within 5 min of exposure to lysozyme, indicating 100% of the spores had the coats removed or had lysozyme-permeable coats. Spores not treated with DTT plus SDS did not exhibit detectable germination in lysozyme. Furthermore, since absence of spore coats may result in a lysozyme germination requirement, colony counts on TSA were verified to enumerate the total population by comparison to direct microscopic counts and counts using TSA with added lysozyme (2 μg/mL). These spores (lacking coat integrity) were not dependent on lysozyme for colony formation. DTT plus SDS-treated *B. cereus* T spores were used within 2 wk of preparation.

Coatless spores of C. *perfringens* 8-6 are dependent on lysozyme for colony formation. *C. perfringens* 8-6 spores were tested for absence of coat by testing the degree of dependence on lysozyme for colony formation. To do so, spores were enumerated by spread plating on egg yolk-containing Tryptose Sulfite Cycloserine (TSC) agar without cycloserine and TSC with or without added lysozyme (2 μg/mL) overlayed with egg yolk-free TSC with or without lysozyme (2 μg/mL) after incubating 16–20 hr at 35°C in an anaerobic jar (BBL Gaspak, Becton Dickinson and Co., Cockeysville, MD) alternatingly evacuated and flushed three times with 10% H_2, 5% CO_2 and 85% N_2 to establish anaerobiosis. TSC was prepared from SFP (Difco) base as detailed by Leininger (1976). The results indicated > 90% of *C. perfringens* 8-6 spores were lysozyme dependent, indicating >90% of the population was lacking the spore coat.

Chlorine dioxide generation and measurement

ClO_2 was generated by the sodium chlorite-acid activation technique as detailed in *Standard Methods for the Examination of Water and Wastewater* (APHA, 1975). Stock ClO_2 solution was stored in the dark at 4°C for use within 2 wk. The ClO_2 concentration was determined immediately prior to use by measuring the absorbance at 357 nm and calculating the concentration from the extinction coefficient of 1250 M^{-1} cm^{-1}. Concentrations determined using this procedure were essentially identical to those determined using the iodometric titration procedure (APHA, 1975). Selected ClO_2 concentrations were made by dilution using 0.05M potassium phosphate buffer at the selected pH value. The concentration of the diluted ClO_2 was verified by absorbance at 357 nm and pH values of diluted ClO_2 were verified.

Spotlessly clean glassware and pipets were used throughout the experiment when in contact with ClO_2.

Experimental reaction system

Spores (ca $1\text{-}2 \times 10^6$/mL unless indicated otherwise) held in an ice-water bath (0°C) were treated with 20, 50 or 80 mg ClO_2/L in 0.05M potassium phosphate buffer at pH 4.5, 6.5, or 8.5. Each population was evaluated using each treatment (pH × concentration), hence nine different survivor curves were generated per population. Samples were removed every 5 or 10 min (1 or 2 min for coatless spores) and ClO_2 was neutralized and/or diluted to an ineffective concentration immediately by addition to a 0.1% peptone (Difco)-water dilution blank or by addition of a few sterile sodium thiosulfate crystals to the sample, each accompanied by vigorous mixing. Chlorine dioxide concentrations in excess of those used in these experiments were verified to be neutralized by these procedures.

B. cereus spores were enumerated by the spiral plating technique (Spiral Systems, Inc., Cincinnati, OH) or by the conventional pour plating procedure. Two to four replicates of each enumeration were done. For either procedure, dilutions were made in 0.1% peptone (Difco) water and enumeration was done using TSA incubated 18–24 hr at 35°C. *B. stearothermophilus* spores were enumerated by either the spiral or conventional pour plating procedure using TSA incubated 72 hr at 55°C. *C. perfringens* spores were enumerated by spread plating using TSC without cycloserine overlayed with egg yolk-free TSC incubated 16-20 hr at 35°C in an anaerobic jar alternatingly evacuated and flushed as detailed above or by using the BBL Gaspak systems to establish anaerobiosis. TSC enumeration medium (basal and overlay medium) for *C. perfringens* 8-6 contained 2 μg freshly prepared, filter sterilized lysozyme/mL.

Testing for ClO_2 injury of *B. cereus* T spores. To determine whether ClO_2 exposure may cause injury, FNA-sporulated *B. cereus* T (designated preparation one) treated with pH 6.5, 50 ppm ClO_2 was enumerated on TSA with and without added lysozyme (2 and 0.2 μg/L), Trypticase Soy Broth (Difco) plus 0.15% agar (Difco), Mannitol Yolk Polymyxin medium without polymyxin (Leininger, 1976), Plate Count Agar (Difco), and TSA without and with added pyruvate (0.66% w/v; Sigma Chemical Co., St. Louis, MO) to scavenge H_2O_2.

Statistical analysis

Survivor curves were fitted by least squares to the non-linear Weibull model. Data were analyzed by analysis of variance using the General Linear Model Procedure (GLM), a packaged program of Statistical Analysis System (SAS Institute, Cary, NC). The Waller-Duncan k-ratio t-test was used to test differences among means.

The Weibull model used in curve-fitting is as follows:

$$N/No = \exp -(time/S)^C$$

where N/No represents the proportion of survivors. C is interpreted as a measure of the constancy of the rate at which organisms die, once they begin dying. C = 1 corresponds to the case in which the rate of death remains constant. A value of C greater than 1 implies that organisms begin to die more and more rapidly as time progresses. S can be interpreted as the point in time when a certain proportion of the organisms are still alive.

In this analysis, N/No = 0.37 was selected as the point at which shoulders on the inactivation curves ended. Unless otherwise specified, the mean time (averaged among all treatment combinations) when the population was reduced 3 log cycles (99.9% inactivation) was used in the analysis to compare ClO_2 resistances among the strains.

RESULTS & DISCUSSION

Since ClO_2 treatment may inactivate and/or injure spores, a procedure which would enumerate injured spores was needed. If ClO_2 reacted with the exterior of the spore, specifically with the spore coat, the spores may be viable but unable to germinate in medium which typically would support germination yet may germinate in the presence of lysozyme (Foegeding and Busta, 1983). Others have indicated benefits of media with added pyruvate or Mannitol Yolk Polymyxin agar and differences among commonly used nonselective media for recovery of injured spores (Johnson et al., 1982). Several media initially were tested for recovery of *B. cereus* T spores. Recovery of ClO_2-treated *B. cereus* T was essentially identical for each medium studied (data not shown). The data indicate injury of the spores by the ClO_2 treatment was unlikely. Enumeration using TSA was selected for subsequent studies.

Figure 1 illustrates representative survivor curves for spores treated with ClO_2 and the curves generated using the Weibull model. For all data, curves generated by this model fit well and were considered an excellent representation of the data. Subsequent figures illustrate, and all comparisons used, the modeled curves only.

A statistical comparison of eighteen survivor curves (2 spore preparations × 3 pH values × 3 ClO_2 concentrations) of *B. cereus* T spores prepared on two occasions using the same sporulation procedure (FNA sporulation) was used to assess the variation among spore populations prepared by one procedure. Although three of nine paired survivor curves (comparing the two populations) had shoulders of different length, overall there was no practical or statistically significant difference in times to inactivate 99.9% of the populations (data not shown). Therefore, subsequent comparison among genera, species, strains and treatments were based upon times to inactivate 99.9% of the populations. To facilitate comparisons, representative data have been presented in this paper. All survivor curves are presented by Hemstapat (1985).

The ClO_2 resistance of spores of the same strain sporulated by two procedures differed significantly ($p < 0.001$) (Fig. 2). *B. cereus* T sporulated in Modified G medium was significantly more sensitive to ClO_2 than when sporulated on Fortified Nutrient Agar. The time the shoulder (lag period prior to

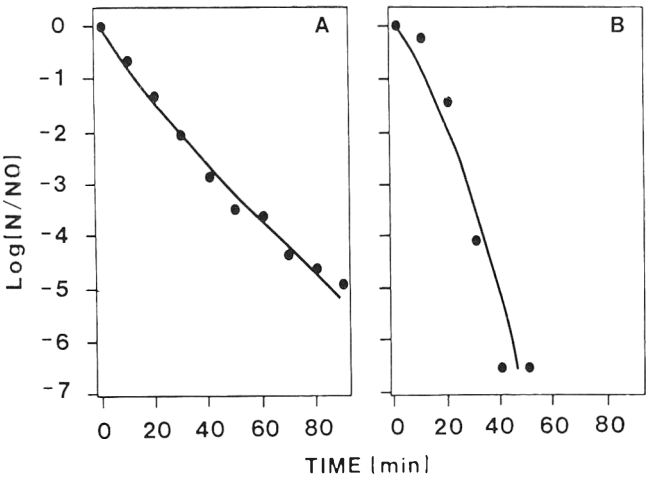

Fig. 1—Representative survivor curves showing the goodness of fit of curves generated using the Weibull Model to the actual data. Log (N/NO) = Log (population at time t/initial population). (A) B. stearothermophilus *ATCC 1518 treated with 20 mg ClO_2/L at pH 4.5; (B) Fortified Nutrient Agar-sporulated* B. cereus *(preparation 1, 8 × 10^6 spores/mL) treated with 50 mg ClO_2/L at pH 8.5.*

rapid inactivation) existed contributed more to the difference in resistance than did the inactivation rate once the shoulder ceased. Modified G medium is a minimal broth medium consisting of yeast extract, $(NH_4)_2SO_4$, glucose, and mineral salts while FNA is a more complex and complete agar medium. These results supported earlier findings that spores produced on different media may show varying resistance to heat or chemicals (Waites et al., 1979; Bayliss et al., 1981). Studies with spores of *C. bifermentans* sporulated on different media showed different responses to inactivation treatments which appeared related to spore structure (Waites, 1982; Waites and Bayliss, 1980; Waites et al., 1980; Bayliss et al., 1981). For example, the more H_2O_2 resistant spores had a thicker cortex and smaller protoplast (Waites et al., 1980).

ClO_2 was an effective sporicide. Significant ($p < 0.001$) concentration effects were seen for all treatment combinations (strain × concentration × pH) as illustrated in Fig. 2 for *B. cereus* T spores. ClO_2 efficiently inactivated bacterial spores under the conditions tested. For example, ClO_2 at 50 mg/L, pH 6.5 reduced large spore populations (ca. 1-6 × 10^6 spores/mL) ≥ 99.9% in approximately 15–38 min and 55 min for *Bacillus* and *C. perfringens*, respectively. Higher ClO_2 concentrations more rapidly inactivated the spore populations. In food plant sanitation, spore populations much less than 10^6/ml would be of concern and would be reduced to an acceptably low level in a shorter time if bacterial and non-bacterial organic loads were sufficiently low.

Chlorine dioxide inactivation of the *Bacillus* spore populations studied was not significantly affected by pH. Although differences due to pH were observed for *Bacillus* spore inactivation, the differences were not consistent among the concentrations for any population nor were they dramatic and, therefore, differences due to pH were not statistically significant. Representative data illustrating the insignificant effect of pH is presented in Fig. 3 where inactivation curves for *B. cereus* F4810/72 and *B. stearothermophilus* ATCC 1518 by 50 mg ClO_2/L at pH 4.5, 6.5, and 8.5 are shown and also is illustrated in Fig. 2 for *B. cereus* T populations. Similar results documenting an insignificant pH effect were obtained with each *Bacillus* spore population studied and at each ClO_2 concentration (20, 50, and 80 mg/L). However, there was a significant pH effect ($p < 0.001$) for *C. perfringens* NCTC 8798 spores (Fig. 4) where more rapid inactivation was achieved at pH 8.5 than at pH 6.5 or 4.5 at each ClO_2 concentration. There was no significant difference in inactivation of *C. perfringens* NCTC 8798 spores between pH 4.5 and 6.5 ClO_2 treatment. Studying vegetative cells of bacteria, Ridenour and Ingols (1947) reported constant bactericidal activity in the pH range 6–10. Benarde et al. (1965) reported no change in the absorption spectra of ClO_2 at pH 4.0, 6.45, and 8.42 indicating dissociation of ClO_2 in that pH range did not occur. However, Benarde and coworkers (1965) did observe faster *E. coli* inactivation at pH 8.5 than at pH 6.5. Data on ClO_2 inactivation of poliovirus 1 and coliphage f2 indicated faster inactivation at pH 9 than at pH 7 and pH 5 (Taylor and Butler, 1982). Since ClO_2 is not hydrolyzed in the pH range studied and there was no pH influence on other spores, pH must affect the spore itself. Differences in the protective mechanism or exterior spore structure may cause the observed pH effect on inactivation. It is tempting to ascribe the observed difference to a generalized difference in aerobic and anaerobic spores; however, only one strain of anaerobic and two species of aerobic spores were tested, so the observed pH effect is based upon very limited data.

Results from the Waller-Duncan k-ratio t-test on the mean time to inactivate 99.9% of the spore populations indicated three distinct groups in regard to ClO_2 sensitivity (Fig. 5). *B. stearothermophilus* ATCC 1518 and MGM-sporulated *B. cereus* T were significantly more ClO_2 sensitive than FNA-sporulated *B. cereus* T (two spore preparations) and *B. cereus* F4810/72. Each *Bacillus* spore population was more ClO_2 sensitive than the *C. perfringens* spore population tested. The group designations were not affected by pH, as illustrated in

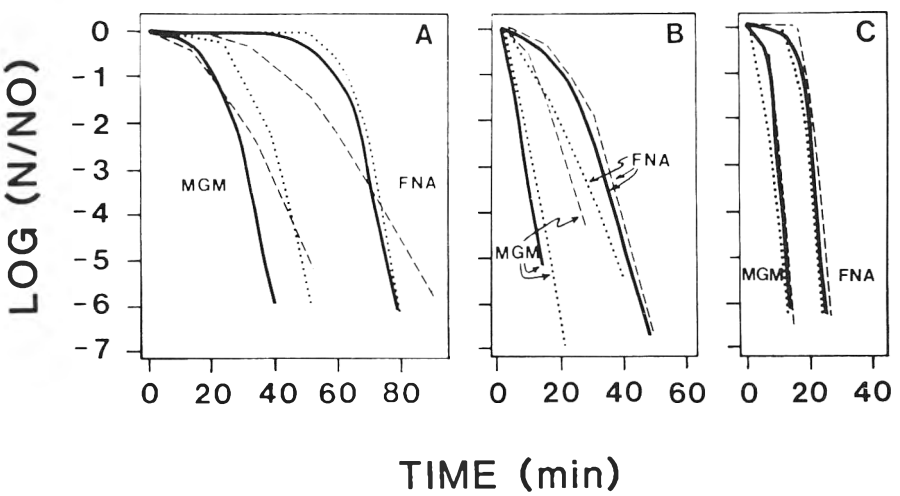

Fig. 2—Effect of sporulation medium on B. cereus *T resistance to ClO_2 and effect of concentration on lethality of ClO_2. Data represent* B. cereus *T sporulated on Fortified Nutrient Agar (FNA, preparation 1, 5 × 10^6 spores/mL) and in Modified G medium (MGM, 2 × 10^6 spores/mL). Sections A, B and C illustrate the effect of 20, 50 or 80 mg ClO_2/L, respectively. Log (NNO) = Log (population at time t/initial population). pH values of 4.5, 6.5 and 8.5 are represented by _____, — — — and · · · · ·, respectively.*

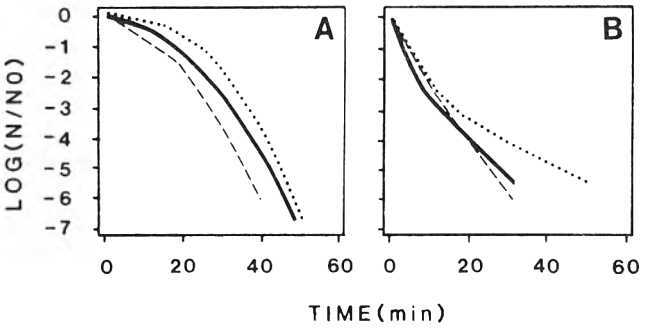

Fig. 3—Insignificant effect of pH (4.5, 6.5, 8.5) on inactivation of Bacillus cereus *F4810/72 (4–8 × 10⁶ spores/mL) (A) and* B. stearothermophilus *ATCC 1518 (B) spores by 50 mg ClO_2/L. Log (N/NO) = Log (population at time t/initial population). pH values of 4.5, 6.5, and 8.5 are represented by _____, — — — and · · · · · · · ·, respectively.*

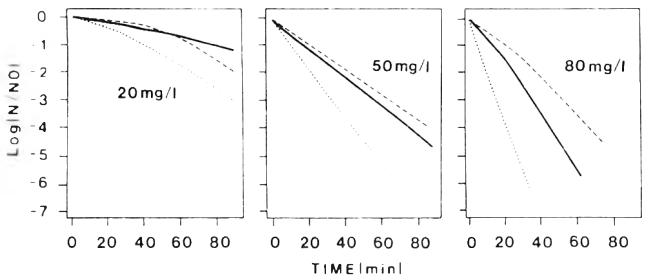

Fig. 4—Effect of pH (4.5, 6.5, 8.5) on inactivation of C. perfringens *NCTC 8798 by 20, 50 and 80 mg ClO_2/L. Log (N/NO) = Log (population at time t/initial population). pH values are represented by _____, — — — and · · · · · ·, respectively.*

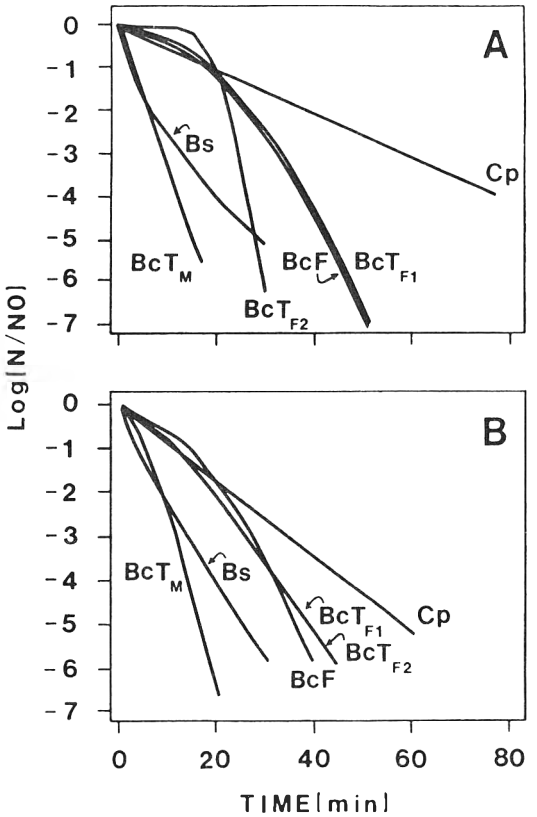

Fig. 5—Comparative sensitivity of Bacillus *and* Clostridium *spore populations to 50 mg ClO_2/L at exposure pH values of 4.5 (Fig. 5A) and 8.5 (Fig. 5B). Data for the following spore populations are shown:* B. cereus *T [FNA preparations 1 (BcT_{F1}), 5 × 10⁶ spores/mL, and 2 (BcT_{F2})],* B. cereus *T [MGM-sporulated (BcT_M), 3.5 × 10⁶ spores/mL],* B. cereus *F4810/72 (BcF, 6 × 10⁶ spores/mL),* B. stearothermophilus *ATCC 1518 (Bs), and* C. perfringens *NCTC 8798 (Cp). Log (N/NO = Log (population at time t/initial population). At pH 8.5, data for FNA-sporulated* B. cereus *T. preparations 1 (BcT_{F1}) and 2 (BcT_{F2}) are essentially identical and are superimposed in this figure.*

Fig. 5 where data for inactivation at pH 4.5 and 8.5 are shown. The pronounced ClO_2 sensitivity observed for *B. stearothermophilus* ATCC 1518 spores was contrary to data compiled by Odlaug and Pflug (1976) which indicate that of six *Bacillus* species compared the thermophile *B. stearothermophilus* is unique in its extreme resistance to hypochlorous acid (the active species of Cl_2 or hypochlorite). Since sporulation medium will affect significantly the sensitivity of the spores, care must be taken to consider sporulation conditions when comparing resistances of different strains. Given this limitation, for the strains and conditions studied, the *Bacillus* spore populations were significantly more ClO_2 sensitive than the *C. perfringens* NCTC 8798 spore population. This is contrary to the bulk of the published literature on hypochlorite inactivation of *Bacillus* and *Clostridium* spores (Ito and Seeger, 1980; Odlaug and Pflug, 1976; Dye and Mead, 1972) which indicates *Clostridium* spores are more sensitive than *Bacillus* spores. However, comparisons are handicapped again since sporulation and handling conditions in these studies differed from those reported in the literature. Our data suggested that, of the strains studied, *C. perfringens* NCTC 8798 sporulated in D-medium would be the best test organism for studies to determine the sanitation effectiveness of ClO_2.

Absence of spore coats or lack of coat integrity markedly increased the lethality of ClO_2 compared to inactivation of spores with intact coats (Fig. 6). These data indicate the important role of the coat in protection of the spore to ClO_2. *B. cereus* T spores lacking coats or coat integrity resulted in survivor curves without shoulders compared to the intact spores. The data suggest that the coat may be responsible for the presence and extent of the shoulder; however, to date the precise basis for the frequent presence of shoulders has not been investigated. Tails were evident on the survivor curves representing *C. perfringens* 8-6 inactivation. The tails indicate <1% of the population had greater ClO_2 resistance which appeared to parallel the initial resistance observed for the parent strain under these conditions. The resistant population probably represents a fraction of the *C. perfringens* 8-6 population with coats and corresponds with the small percentage of the population which was able to form colonies on TSC without lysozyme.

The spore coat is important for protection of spores against hypochlorite and hydrogen peroxide inactivation (Wyatt and Waites, 1973, 1975). The coat appears to act as a permeability barrier to chlorine and other agents. The effectiveness of the coat at excluding ClO_2 may be responsible for the extent of the shoulder observed in survival curves. Removal of spore coat proteins does not affect spore viability but renders spores more sensitive to lysozyme germination, hydrogen peroxide and hypochlorite (Wyatt and Waites, 1975; Waites and Bayliss, 1979; Bayliss et al., 1981; Waites, 1982; Foegeding and Busta, 1983). These data suggest a two-step sporicidal treatment, including one step to weaken the coat (such as alkali treatment, as suggested by the observed pH effect on *C. perfringens* inactivation by ClO_2), followed by treatment with ClO_2 may be highly effective for sanitation. When *Bacillus* spores were treated with NaOH (pH ca. 13), which solubilized alkali-soluble protein presumably from the spore coat, they were even more sensitive to lysozyme and hydrogen peroxide treatment (Gould et al., 1970). NaOH has been shown to alter the structure of the spore coat (Duncan et al., 1972; Waites et al., 1976). The literature suggests that at least two defined spore components (disulfide rich protein and alkali soluble pro-

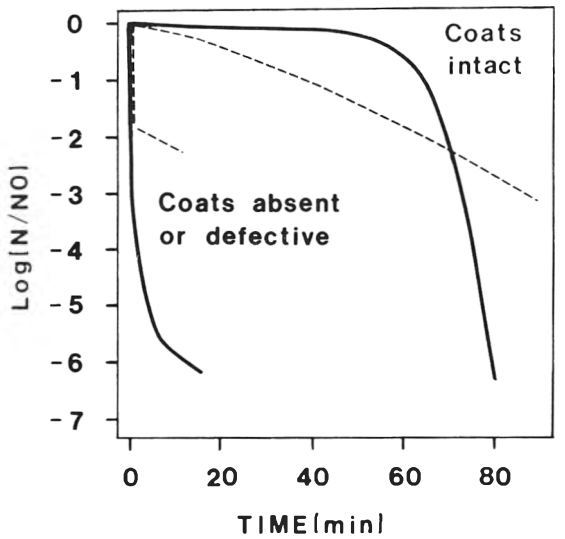

Fig. 6—Effect of coat on ClO_2 inactivation (20 mg/L, pH 8.5) of Bacillus cereus *(_____) and* Clostridium perfringens *(— — — —) spores. Data represent spore populations of* B. cereus *T (NO = 10^6 CFU/mL),* B. cereus *T treated to remove coat proteins (NO = 10^6 CFU/mL),* C. perfringens *NCTC 8798 (NO = 10^6 CFU/mL), and* C. perfringens *8-6 (NO = 5×10^5 CFU/mL). Strain 8-6 is a coatless mutant of NCTC 8798. Log (N/NO) = Log (population at time t/initial population).*

tein) exist and are involved in permeability. This may explain the enhanced inactivation of *C. perfringens* NCTC 8798 at pH 8.5 (Fig. 4). Enhanced activity of ClO_2 at basic pH values may be related to base hydrolysis or deprotonation of *C. perfringens* coat proteins. Although we found both *Bacillus* and *Clostridium* spores which lacked coats or had disrupted coats to be significantly more sensitive to ClO_2 than spores with intact coats, studies with other sanitizers have shown that the spore coat might exert a protective barrier in one sporeformer but not in another (Bayliss and Waites, 1976; Waites and Bayliss, 1979).

This study documents the effective inactivation of both *Bacillus* and *Clostridium* spores by ClO_2. It was not designed to illustrate or recommend application conditions since in sanitation applications smaller spore populations than were studied in this work and presence of other organic or inorganic debris would be encountered. ClO_2 currently is used with success in water treatment and other situations although documentation of spore inactivation had been limited.

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Influence of Almond Harvest, Processing and Storage on Fungal Population and Flora

A. DOUGLAS KING, JR. and JOHN E. SCHADE

ABSTRACT

The fungal population and flora of almonds were studied from receiving at the processing plant through processing and storage. Late harvest almonds had lower counts than those from early or mid season. The highest quality processed nutmeats had the lowest mold population while the lowest quality had the highest population. A significant drop in the population occurred during storage at 25°C but not at 5°C. *A. niger* group and *P. glabrum* were the most prevalent of the 12 genera and 51 species identified. During storage some molds survived better than others. *A. niger* group survived while the proportion of *Cladosporium* and *Pencillium* dropped.

INTRODUCTION

ALMOND PROCESSING starts with removal of the hull, usually at or near the farm. At the processing plant the shell is removed leaving the almond meat. The nutmeats, kept separate by variety, are graded for size and quality by physical, electronic, or visual-hand separations. After processing, the predominant product is whole almonds. During processing the whole, acceptable nutmeats are separated from those having mechanical, physiological, or insect damage. The latter are further segregated into edible and inedible fractions. The remaining edible almonds go into manufactured almond products such as sliced or diced almonds, almond paste, and blanched almonds. The inedible fraction is often extracted for almond oil.

The fungal flora of almonds is not well documented. Several studies have been conducted on the bacterial flora of almonds (Ostrolenk and Hunter, 1939; King et al., 1968; Kokal and Thorpe, 1969; King et al., 1970). Fungal studies on almonds have been related to organisms causing hull rot (Mirocha and Wilson, 1961), to *Aspergillus flavus* and related organisms (Phillips et al., 1976; 1979; Blaser and Schmidt-Lorenz, 1981), or to osmotolerant yeasts (Windisch et al., 1978).

The variability of fungi or conidia on dry foods has not been extensively studied. Beuchat (1979) studied the survival of *A. flavus* on dried foods at water activity (a_w) less than 0.78. He reported that the survival of conidia was greatest at low a_w and that low pH in acid foods became detrimental to conidia with increasing storage time at 21°C. The a_w of retail packaged almonds is in the range 0.47–0.50 (Beuchat, 1978; King et al., 1983).

This research was initiated to determine the fungal population and flora of almonds and the influence of processing and storage on them. The effect of storage at different times and temperatures on fungal viability on a dry food was measured.

MATERIALS & METHODS

NONPARIEL ALMONDS (*Prunes amygdalus*) were collected from a processing plant at three periods during the harvest season; early, mid, and late season. At each sampling time almonds were selected from incoming nutmeats received from the San Joaquin and Sacramento valleys (principle growing areas). During processing different processing fractions were selected at the three sampling times. Almonds removed from the processing stream for further processing and those remaining in the processing stream were selected. The identification and number of samples selected are indicated in Table 1, representing as evenly as possible the three sampling periods. In some cases the desired samples were not available. Fines that pass a no. 12 seive following dicing whole almonds were also collected and analyzed.

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Table 1—Mold counts on whole almonds of various processing categories

Sample description	Mean $\log_{10}$ count/g	SEM[a]	No. of samples
Incoming nutmeats (north)	4.17	0.128	14
Incoming nutmeats (south)	4.12	0.117	11
Blanched whole almonds	2.03	0.333	3
Blanched rejects	2.64	0.430	3
Manufacturing stock	4.22	0.117	4
Rejects	4.52	0.102	7
Shriveled rejects	4.59	0.116	3
Select whole nutmeats	3.48	0.176	2
Fines from diced almonds	5.18	0.594	4

[a] Std. error of mean

Table 2—Genera (or group) of molds identified on plates from enumeration studies

Genus or group	% of all colonies	% of samples with organism
Alternaria	1	17
Aspergillus (other than niger)	1	45
A. niger group	35	90
Cladosporium	8	50
Penicillium	6	58
Rhizopus-Mucor	9	70
Yeast	19	58
Unidentified	20	73

The almonds were analyzed the day after samples were collected and subsequently after storage at either 5°C or 25°C, two common temperatures for commercial storage. Samples for fungal counts were prepared by adding 450g sterile water to 50g almonds and "stomaching" (sample and diluent in a plastic bag are pummeled by two external paddles) for 2 min (Sharpe and Jackson, 1972). Appropriate dilutions were made in 0.1% peptone water and the samples were plated by spreading 0.1 mL on the surface of dichloran-rose bengal-chlortetracycline agar (DRBC) (King et al., 1979). Occasionally dichloran-glycerol agar (DG-18) (Hocking and Pitt, 1980) was used to isolate osmophilic organisms. These two media were used because they control the growth of rapidly spreading molds that otherwise overgrow the plates. Plates were incubated upright at 25°C for 5 days before counting and isolating mold colonies for identification.

Isolated cultures were identified according to standard taxonomic schemes (Raper and Thom, 1949; Raper and Fennell, 1965; Ellis, 1971; Simmons, 1967; and Gilman, 1945). Names of the *Penicillium* species have been changed to conform with those accepted by Pitt (1979). Water activity was measured with a Beckman Hygroline instrument standardized against salt slushes. Almonds in glass jars with salt slushes used to adjust a_w (Stoloff, 1978) and held at 25°C (King et al., 1983) were plated at intervals up to 1 month.

Statistics were performed on population data transformed by $\log_{10}$ and reported as such. The least significant difference test reported for 0.05 probability (LSD) was used to compare means. All statistical tests and analyses reported can be found in Steel and Torrie (1960).

Table 3—Recovery of molds from almonds at different periods during study[a]

Mycoflora identified	Receiving and processing	0 Days	Storage days: 136	210 (5° Storage)	385	136	210 (25° Storage)	385
Alternaria alterneta	X			X	X		X	
Aspergillus niger group								
A. aculeatus	X			X				
A. ficuum	X			X				
Aspergillus flavus group								
A. flavus	X	X	X	X	X	X		
A. parasiticus				X				
Aspergillus glaucus group								
A. amstelodami			X	X				
A. chevalieri				X				
A. montevidensis				X			X	
A. pseudoglaucus	X							
A. repens							X	
A. ruber				X				
Aspergillus carneus		X						
Aspergillus rugulosus							X	
Aspergillus silvaticus				X				
Aspergillus terreus		X						
Aspergillus ustus		X					X	
Aspergillus versicolor			X					
Aspergillus wenti	X	X	X					
Cladosporium cladosporioides		X					X	
Cladosporium herbarum							X	
Epicoccum nigrum				X				
Fusarium spp.	X						X	
Mucor circinelloides							X	
Paecilomyces variotii	X	X		X				X
Penicillium brevicompactum	X							
Penicillium chrysogenum				X				
Penicillium citreonigrum			X					
Penicillium citrinum	X	X		X				
Penicillium corylophilum	X			X				
Penicillium crustosum	X							
Penicillium echinulatum		X						
Penicillium glabrum	X	X	X	X				
Penicillium purpurogenum		X					X	
Penicillium restrictum							X	
Penicillium sclerotiorum		X		X				
Penicillium spinulosum	X			X				
Penicillium sublateritium				X				
Penicillium variable				X				
Penicillium viridicatum		X						
Rhizoctonia solani								X
Rhizopus stolonifer							X	
Syncephalastrum racemosum				X				
Ulocladium alternariae	X							
Ulocladium atrum					X			X
Ulocladium botrytis							X	
Ulocladium chartarum		X						
Ulocladium lanuginosum					X			

[a] X indicates the mold was isolated from almonds at the time and temperature indicated.

RESULTS

Counts on whole nutmeats

The water activity of almonds under normal handling and marketing conditions is too low for microbial growth. The almonds tested in this research had an a_w of 0.46–0.48. Hence, the counts and molds isolated in this work reflect nongrowing flora; any changes in flora composition during storage are due to stability differences amongst the molds.

Samples were collected in September, November, and December to determine the influence of time of harvest on mold population and flora. Mean $\log_{10}$ mold population/g (MLM) on unprocessed nutmeats were 4.41, 4.27, and 3.72 for these periods representing early, mid, and late harvest times. Only the MLM for late harvest were significantly lower when tested by the least significant difference test (LSD 0.05). Early and mid season harvest counts were not significantly different.

Table 1 lists the counts obtained from almonds received at the processing plant and during processing immediately following delivery. Samples of incoming nutmeats were classified as originating from north or south of Sacramento, CA in order to compare counts on nutmeats from these two principle almond growing regions. There was no significant difference between the MLM for nutmeats from the north or from the south. There was evidence, however, that processing removes some of the mold contamination from the incoming nuts, possibly by dust removal. Whole select nutmeats, the highest quality kernels, had a MLM that was that significantly lower (LSD 0.05) than the MLM of the incoming nutmeats from either the north or the south. A comparison of the MLM on the different categories of almonds obtained during processing also shows that processing tends to separate nutmeats with different mold populations (Table 1). For example, the MLM for the rejected nutmeats was significantly higher (LSD 0.05) than that for the select whole nutmeats. Similarly, although not significant, the edible damaged nutmeats designated for manufacturing use had a somewhat higher MLM than that of the whole select nut-

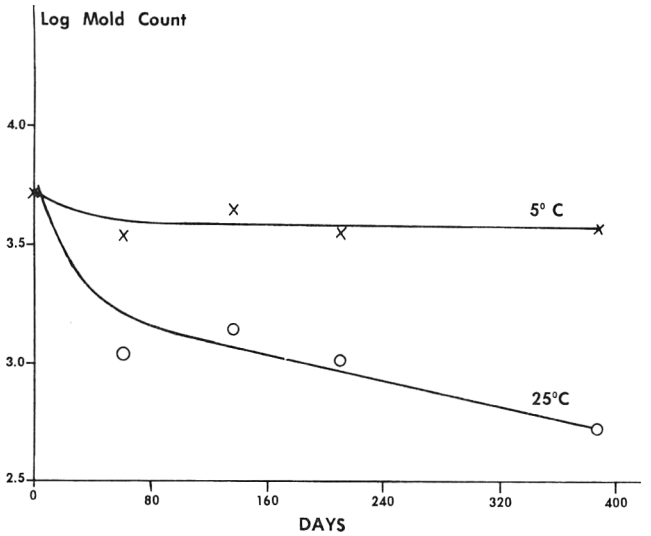

Fig. 1—Mold counts (log_{50}/g) for almonds stored at either 5°C or 25°C for various periods of time.

meats. Blanched almonds had the lowest population. The rejected nutmeats, which normally are extracted for oil, had the highest population.

After dicing, almond pieces are screened to remove the very small pieces (fines). The fines contain a large proportion of the outer skin of the almond kernel (pellicle). The large population on these fines is probably because of the concentration of the mold spores on the outer portion of the almond. The mold flora consisted primarily of *Rhizopus arrhizus* and *R. stolonifer* and *Aspergillus niger* gp., but *Penicillium chrysogenum, Aspergillus terreus* group, *Cladosporium* spp., *Aspergillus flavus* group, and yeasts were also frequently isolated.

Population after storage

The fungal population was determined periodically on almonds stored at 5° and 25°C for up to 385 days. When all the MLM data for sample categories and storage periods were pooled, the samples stored at 25°C had a significantly lower population (LSD 0.05) from the beginning of the study to the end, and the final count was significantly lower than the population at 5°C (Fig. 1). There was no significant drop in population of the almonds stored at 5°C. The large drop in mold population occurred within the first 60 days of storage at 25°C. The population, 60 days and afterward, was significantly lower (LSD 0.05) than the original fungal population. These values indicate the importance of temperature on the survival of fungi in a dry environment where growth cannot occur.

Fungal flora isolated from almonds

When colonies were counted, a preliminary identification was made of the flora on the basis of the gross colony appearance. Eight groups were routinely identified (Table 2). Each genus (or group) identified for the entire study is listed as a percentage of the total number of colonies. Also listed is the percentage of samples containing each genus or group. *A. flavus* colonies were identified in 30% of the samples.

After colony counts and preliminary identification of colonies by gross appearance were made, fungal colonies were picked from plates for purification and identification. Colonies were selected that represented the flora of the sample examined. A total of 159 fungal isolates from the almonds were identified. These represented 12 genera and 51 species of molds (Tables 3 and 4). The most numerous isolates were aspergilli (20 species) and penicillia (16 species). Less than five species were identified from any other genus.

The most prevalent of all fungi were members of the *A. niger* group, usually *A. aculeatus* and *A. ficuum* (Table 2). Colonies thought to be *A. flavus* were isolated and identified more selectively than other members of the genus because of the importance of the metabolite aflatoxin. The most frequently isolated species of the genus *Penicillium* was *P. glabrum*. *R. stolonifer* was frequently isolated and was nearly always present.

The flora isolated from DRBC agar plates reflects only a portion of the total mycoflora. Because of the high a_w of DRBC, a number of molds that require a relatively low a_w for growth, or those that grow better at low a_w, will not be counted and identified on DRBC. Use of medium of lower a_w, like DG-18, enables growth of a different mycoflora, and thus these organisms could be isolated and identified.

Flora changes during storage

There was a change in percentage of some colony types across storage time and no change with others, indicating differences in stability. As a result, the proportion of *A. niger* group colonies increased with time on almonds stored at 25°C (Table 5). This increase was linear and significant (LSD 0.05) with a correlation coefficient of 0.92. There did not seem to be a similar trend with the other aspergilli. With penicillia on almonds stored at 25°C, there was a significant drop (LSD 0.05) in the percentage of colonies. The percentage of *Cladosporium* colonies also dropped at 25°C from the early counts to day 60 and thereafter.

Some samples had a mixture of several molds while other

Table 4—Mycoflora isolated after incubation of almonds at different a_w and different times up to 1 month at 25°C[a]

	a_w					
Identified mycoflora	0.98	0.93	0.90	0.84	0.81	0.75
Alternaria alternata			X			
Aspergillus glaucus group						
A. amstelodami				X		
A. chevalieri					X	
A. mortevidensis				X	X	
A. cristatus		X				
A. repens				X	X	X
A. ruber		X		X		X
A. umbrosus				X		
Aspergillus aculeatus	X					
Aspergillus flavus			X	X		
Aspergillus wentii			X			
Cladosporium cladosporioides	X					
Penicillium glabrum			X	X		
Penicillium implicatum	X		X	X		
Penicillium rugulosum						X

[a] X indicates the mold was isolated from almonds under the conditions ind cated.

Table 5—Percent distribution of colonies identified from enumeration studies on almonds after different storage times at 5°C or 25°C

Storage temp °C	Time	Total no. of colonies for 8 samples	Percent							
			Aspergillus[a]	*A. niger*	*Penicillium*	*Cladosporium*	*Alternaria*	Yeast	*Rhizopus*/ *Mucor*	Other
5°	0 days	481	1	16	13	30	0	9	3	27
	60	637	1	17	3	4	0	0	70	6
	136	226	3	32	5	0	8	20	22	11
	210	1424	1	20	10	8	1	10	4	46
	385	1863	1	40	3	17	3	21	1	15
25°	0	877	1	27	10	26	6	13	5	18
	60	321	3	49	4	1	0	10	9	25
	136	139	4	35	2	1	0	8	49	1
	210	479	1	52	2	5	1	3	12	26
	385	464	8	67	1	0	1	15	2	7

[a] Other than *A. niger* group.

samples had a large proportion of a particular kind of mold. This large variation in the percentage of colonies resulted in a large estimated variance making it impossible to predict trends in survival of many types of molds whether or not the angular transformation for percentage data was used. It appears, however, that there was a decline in the proportion of several kinds of colonies during storage at 25°C. This decline seems related to the proportional increase in *A. niger* colonies, which apparently were more stable. Trends were less obvious with 5°C storage experiments.

To get a better assessment of the total mycoflora, almonds were incubated at several a_w for up to 1 month at 25°C and then plated for flora isolation and identification. This storage encouraged development of the particular mycoflora that could survive or grow at the selected a_w. The predominant mycoflora obtained at each of six a_w are shown in Table 4. With almonds incubated at a_w of 0.84 or below, a different set of fungi were isolated than from almonds incubated at high a_w, 0.90 or above. *Alternaria alternata, Cladosporium cladosporioides* and *A. aculeatus* were only isolated from almonds equilibrated at a_w of 0.90 or above, while the *A. glaucus* group of fungi were most frequently isolated at a_w of 0.84 or below. Visible mold growth on almonds has been reported to be related to time and a_w at a given temperature. The lower the a_w, the longer the time before mold becomes visible (King et al., 1983).

DISCUSSION

THE EFFECT of processing on fungal counts is clearly shown by the data in Table 1. Each processing step seems to separate almonds with different amounts of fungal contamination. The highest quality nutmeats had significantly lower population (LSD 0.05 on $\log_{10}$ means) than those on unsorted incoming nutmeats or those on inedible rejected nutmeats. The highest quality nutmeats also tended to have lower counts than the manufacturing stock, which consists of damaged edible nuts destined for use in manufactured products. It was not surprising to find that counts on manufacturing stock, which includes some insect damaged nuts, were higher than those on the highest quality whole nutmeats, since King et al. (1970) found yeast and mold counts increase with insect damage. The highest counts in the present study were on the fines separated from the cutting operation, illustrating that the fungal flora is primarily on the external surface of the nutmeat. This observation is in agreement with the observation of King et al. (1970) that softshell nutmeats have higher fungal counts than hardshell nutmeats, because softshell nutmeats are open during harvest and subject to contamination with airborne fungal spores.

Mold colonies obtained by plating any naturally contaminated product such as almonds may be derived from hyphal fragments, asexual spores or sexual spores. Since the almonds in this study were generally sound, dry, and free of active mold growth, it is likely that the major proportion of any particular mold count was derived from asexual spores, the primary means of mold dispersal. Thus, any difference in survival amongst the molds composing the flora was probably due to a difference in stability of their asexual spores (e.g., conidiospores, sporangiospores) and not to a difference in the composition of the mold propagule, i.e., hyphal fragment versus conidiospore.

Almonds have a varied fungal flora as indicated by the 51 species in 12 genera isolated and identified. The two genera with the largest number of isolates were *Aspergillus* and *Penicillium* as is the case with many processed dry foods. The use of two media of different water activity show that the flora isolated depends upon the medium used.

Relatively little has been published about the longevity of molds on dry foods, ie., the viability of mold propagules after storage on dried foods at various temperatures. Some studies have been made of the survival of specific molds (conidia) on dried foods, but little has been done to define the changes in flora that can be expected to occur during storage if there is a difference in survival amongst the molds in the conidial flora of a given product. In addition to length of storage, temperature of storage and water activity of the product are important factors affecting survival of molds on foods that are sufficiently dry to prevent mold growth. Dry conditions appear to favor survival of molds (Beuchat, 1979); survival appears to decrease as available moisture increases toward the water activity limiting growth. Similarly, low temperatures that limit growth and metabolic activity (e.g., 5°C in this study) seem to favor mold survival, while temperatures that favor growth and metabolic activity (e.g., 25°C in the present study) tend to limit mold survival on dry foods. Only a few types of mold diminished noticeably with storage at 25°C, while a wide variety of molds persisted on almonds for the full storage period of 385 days, even at 25°C. These temperature-time changes were consistent with both population data and survival of the flora. The implication of the change in flora with time is that a food could have a different proportion of fungi after storage than before. This work clearly shows that molds can survive for a long time on foodstuff stored under normal conditions. Thus the possibility of spoilage or mycotoxin production is always present should abuse of good storage conditions occur.

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Dehydration and Separation of Grape Pomace in a Fluidized Bed System

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ABSTRACT

Grape pomace components (seed and skin) were separated in a fluidized bed system based on their aerodynamic behavior. The Weibull equation was used to explain the elutriation history. Seed dehydration was characterized by a first period of constant drying rate and a second period in which drying rate decreased following Fick's liquid molecular diffusion model. The order of magnitude of heat and mass transfer parameters, both internal and external, was similar to those reported in the literature.

INTRODUCTION

THE GRAPE INDUSTRY has increased significantly in Mexico during recent years. The area destined to grape harvest increased from 12,000 ha to 55,750 ha in the period 1960–1980. Agricultural yield during the last ten years was 9.9 ton of grape/ha. This industry generates a great volume of waste-material which could be utilized for obtaining products of commercial interest. In 1980, 550,000 tons of grapes were produced from which 65% were destined to industry: 35,760 tons for wine making, 301,730 tons for brandy, and 20,020 tons for dehydrated grapes. This 65% produced approximately 47,000 tons of red grape pomace.

Red grape pomace (12 kg/100 kg fresh grapes), constituting seed and skin, could be an important source for obtaining products of industrial interest: alcohol (50 g/kg pomace), anthocyanins (167 mg/kg pomace), tartrates (8.3 g/kg pomace), oil (33.3 g/kg pomace), tannins (10 g/kg pomace) and protein (20 g/kg pomace) as reported by Fantozzi and Betschart (1979).

At the present time there are grape seed oil processing plants in Italy, the Soviet Union, Hungary, Spain, France, West Germany, Argentina and the United States (Kinsella, 1974). The most common process employed in pomace separation and seed dehydration, previous operations to oil extraction, consists of the following stages: (1) light drying of the product, a stage in which the high humidity present, due to the acid extraction of tartrates, is reduced to allow pomace components separation; (2) pomace screening for separating skin and seed; the separation is completed with the use of air currents that blow out the skin particles joined to the seed; (3) final seed dehydration in a rotary dryer obtaining a product with a moisture content between 8 to 10g H_2O/g dry solid (Amerine et al., 1984). The technological alternative analyzed in this work establishes the possibility of separating the grape pomace and dehydrating the seed simultaneously in a fluidized bed system.

The purpose of this work was to determine equilibrium conditions of grape seed and drying air at different temperatures and relative humidities. From this information drying curves were analyzed to obtain the heat and mass transfer kinetic parameters during dehydration. Finally, the separation kinetics of grape pomace components was studied and a mathematical model for explaining skin elutriation as a function of time was developed.

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MATERIALS & METHODS

Apparatus

This work was carried out in the experimental set up outlined in Fig. 1. In this system ambient air was supplied by the compressor (A) which services the laboratory. Air was circulated through an ice trap (B) which diminished the excess humidity present and eliminated oil traces. A pressure regulator (C) maintained the system pressure constant at 5 kg/cm^2. Air was sent to a system of two parallel columns packed with silica gel (D). These two columns maintained a low humidity level in the air (approximately 0.002g H_2O/g dry air). A rotameter (E) with a maximum capacity of 20 m^3/hr at 5 kg/cm^2 and 20°C was employed for controlling air supply to the bed fluidized system. Temperature was controlled with a rheostat connected to a 500 watt heating resistance (F) coiled on a tube at the packed column outlet.

From the heating section, air was fed to the fluidized bed column (G), which consists of three main parts: feeding section, fluidizing chamber and a disengaging section. In the feeding section there was a distributor which comprised a 1 mm wide aluminum perforated slab. A square arrangement with a 0.5 mm orifice diameter and 5 mm pitch was employed. A temperature and pressure cell was situated before the distributor. This cell had two perforations: one communicated to a manometer to read the pressure loss in the column and the other allowed feed air temperature to be measured by the insertion of a thermocouple.

The fluidizing chamber was a 480 mm column with 29 mm internal diameter. The distributor was located at the lower end of the column and at the upper end stands the cyclones. A differential manometer (H) connected to both ends measured the pressure loss in the chamber. On the top of the chamber the air flow could be diverted to a temperature cell where dry and wet bulb temperatures were measured. Feeding air dry and wet bulb temperatures could also be measured in this cell.

Finally, the third section consisted of a system of cyclones (I) constructed from 1L Erlenmeyer flasks and adapted with a tangential entrance and an internal baffle. The function of the cyclones was to

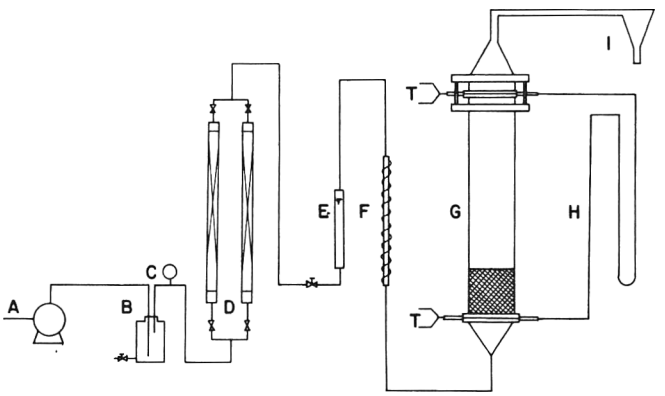

Fig. 1—Experimental apparatus: air compressor (A); water-oil trap (B); pressure controller (C); packed columns (D); flowmeter (E); heating section (F); fluidized bed (G); differential manometer (H); cyclones (I); thermometer (T).

collect the elutriated solids during the operation. To avoid heat losses through the equipment walls, the chamber was isolated with glass fiber achieving adiabatic operation conditions.

Desorption isotherms

Fresh product desorption isotherms were determined in humidity cells (McCune et al., 1981) containing saturated salt solutions in order to produce specific constant relative humidities (Brooker et al., 1974).

For this determination, cells were placed in a constant temperature chamber. Samples were weighed periodically until they reached equilibrium. Samples were considered to be in equilibrium when they showed a constant weight in the first three decimal figures during two consecutive days. Moisture was determined in a vacuum oven at 70°C and 48 mm Hg during 24 hr (AOAC, 1980). Isotherms were determined at 32°, 49° and 68°C.

Pomace conditioning

Grape pomace has a strong tendency towards stickiness because of its high residual carbohydrate content which makes the separation of seed from skin difficult. To overcome this problem, pomace samples were washed with hydrochloric acid solutions, with concentrations between 0.5 and 5%. It is important to point out that this acid treatment favors anthocyanin and tartaric acid extractions (Metevier et al., 1980; Amerine et al., 1984). Washing consisted of two stages: (1) washing with three volumes of a 1% HCl solution during 4 hr and draining; (2) washing with two volumes of a 0.5 HCl solution during 12 hr and draining. Lower acid concentrations did not avoid stickiness problems during pomace separation and higher acid concentrations resulted in product cluster due to organic matter degradation.

RESULTS & DISCUSSION

Desorption isotherms

Grape seed desorption isotherms at 32°, 49°, and 68°C are shown in Fig. 2. Each point represents the mean value of three determinations. The experimental curves show the sigmoidal behavior of type III isotherms (Labuza, 1968).

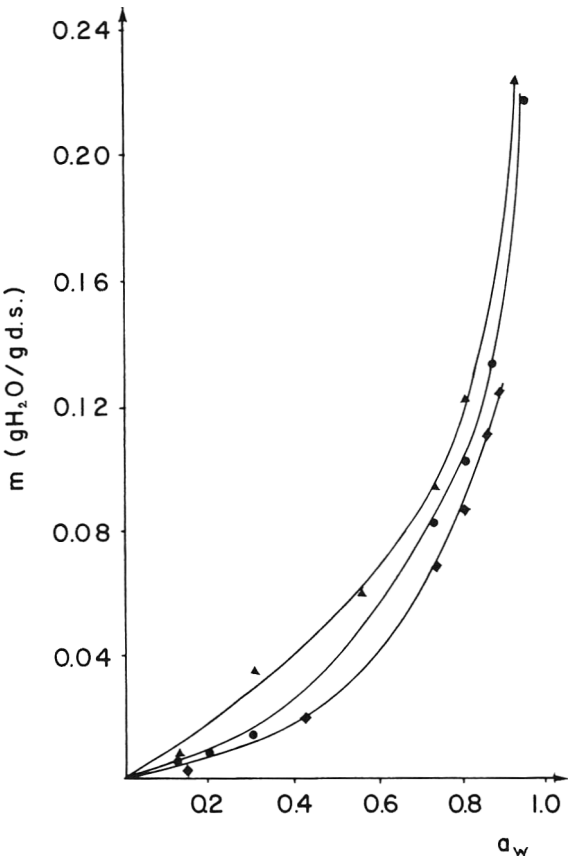

Fig. 2—Grape seeds desorption isotherms: ♦ T = 68°C; ● T = 49°C; ▲ T = 32°C.

From the magnitude of equilibrium moisture contents it can be noticed that seed composition, rich in cellulosic material, gives this product a low hygroscopicity in relation to other materials which have higher simple carbohydrate concentration.

The data obtained were modeled mathematically by means of Henderson equation (Iglesias and Chirife, 1976):

$$(1 - a_w) = \exp(-Jm^n),$$

where a_w is the product water activity, m is the corresponding moisture content, and J and n are equation constants. This model explained adequately the isotherms behavior in the whole relative humidity range under study as can be seen in Fig. 2. Values of Henderson equation parameters, J and n, for each isotherm are given in Table 1.

B.E.T. isotherm model (Labuza, 1968) was linearized to fit experimental values at low moisture contents:

$$\frac{a_w}{(1 - a_w)m} = \frac{1}{m_m F} + \frac{a_w(F - 1)}{m_m F}$$

where m_m is the single layer moisture content and F is a constant for the particular temperature and gas-solid system. From this analysis, the single layer moisture content at 32°C was obtained: 3.34 kg H_2O/100 kg dry matter.

Separation

The separation of grape pomace components in a fluidized bed system was analyzed on an aerodynamic basis. The effect of superficial velocity length:diameter ratio and temperature on separation kinetics was studied. The procedure for determining work intervals for each one of these variables is detailed below.

Superficial velocity. To analyze the possibility of achieving separation by means of a fluidized bed system, the different minimum fluidization velocities of each component and their apparent densities have to be determined. In this case apparent densities of dry seed and skin were 1.176×10^{-3} and 0.425×10^{-3} kg/m^3, respectively. Minimum fluidization velocities were determined separately for each component both in wet and dry states. Results obtained are presented in Table 2; humidity content of the product is indicated in each case. It can be observed that there were important differences between the behavior of wet and dry solids. The reason for this difference was the strong dependence of the solids apparent density with its moisture content. It can also be seen that due to the hygroscopic capacity of the wet skin, this component has a higher minimum fluidization velocity. Work air superficial velocity range was defined between dry seed minimum superficial velocity: $u_m = 3.4$ m/sec and dry seed elutriation velocity: 5.9 m/sec.

Table 1—Values of Henderson equation parameters: J and n for grape seed desorption isotherms

Temperature (°C)	J	n
32	12.91	0.95
39	8.92	0.68
68	7.20	0.64

Table 2—Grape pomace components minimum fluidization velocities at wet and dry states

Grape pomace component	Moisture (g H_2O/g dry solid)	Minimum superficial velocity, u_{mf} (m/sec)
Seed	0.084	3.4
	0.85	4.0
Skin	0.135	1.7
	1.40	5.0

Temperature. The work temperature range was determined between ambient temperature: 25°C and the temperature at which the seed fat content did not show oxidation: 65°C (Peraza, 1983).

Length:diameter ratio. Preliminary tests demonstrated that at a fluidization velocity of 5.0 m/sec bed length could not be increased as much as four times the tube diameter without the solids showing plug behavior during dehydration. On the other hand, the minimum length at which the bed was uniformly fluidized was 0.5 times the bed diameter.

With the preliminary information obtained, separation and dehydration tests were carried out separately. The experiments developed and their conditions are listed in Table 3.

The effect of operation variables:fluidization velocity and length:diameter ratio upon the separation of grape pomace components was studied in this work. Temperature did not have a significant influence on skin elutriation (Peraza, 1983).

Results obtained are presented in Fig. 3 and 4. It can be observed that for a specific length:diameter ratio an increase in fluidization velocity led to an increase in separation rate. Alternatively, at a constant fluidization velocity, an increase in the length:diameter ratio decreased separation rate. These results suggest the following behavior: an increase in air superficial velocity results in a more vigorous bed fluidization, and, therefore, particle interaction is more frequent. Because of this phenomenon, a particle size decrement occurs (attrition effect) which favors skin detachment from the seed and its eventual transportation. Simultaneously, as the superficial velocity increases, the drying rate increases, leading to a faster skin weight loss with time favoring skin elutriation. With regard to length:diameter ratio it was noted that an increment in bed length, i.e. an increment of skin in the fluidization chamber, retards separation rate.

Profiles of experimental curves, as shown in Fig. 3 and 4, suggest that the separation phenomenon is not only a function of material elutriation but also depends upon solid drying occurring at the same time. For this reason, equations proposed by Leva (1959) to model mathematically elutriation did not satisfy experimental data. Detailed analysis of these curves suggested that at the beginning of transportation, the skin has a high moisture content and a relatively high density. Furthermore, at this stage, the skin was not completely free in the bulk of the bed but partially attached to the seed. This situation explains why at the beginning of the operation there is a lag in skin elutriation, and separation curves tend to stay in a region of approximately $C(t)/Co = 1$. As experimental time proceeds, it can be observed that the separation rate increases until it reaches a maximum. This behavior could be explained in terms of the simultaneous drying occurring in the chamber which lowers skin density favoring its detachment from the seed and, thus, its transportation. Finally, in the lower part of the curve a decrease in elutriation rate can be observed because the dehydrated pomace has not reached the degree of attrition and separation needed which would result in the total elutriation of the skin. Therefore, in general terms, it is proposed that during the first stage of the operation, transportation is controlled by the drying rate and during the second stage by mechanical effects.

Based on this behavior, alternative models to the Leva equation were discussed. The model to be developed should consider the simultaneous effect of drying and separation during the experimental operation. Survival models were tested for this purpose: log-normal, gamma and Weibull functions. From these models, the Weibull equation is relatively easier to use because of the low number of parameters involved. Weibull distribution function (Gross and Clark, 1975) is defined by the following equation:

$$\frac{C(t)}{Co} = \exp\,(-t/\theta)^b$$

where $C(t)/Co$ is the skin fraction in bed at any time, and $(1/\theta)$ and b are the scale and form parameters, respectively. Additionally, Weibull model includes the equation proposed by Leva (1959) in a particular case: when the form parameter b is equal to 1.

Based on these considerations the proposed model was first fitted by linear regression. Results obtained indicate a lack of fitness towards the last stages of skin elutriation. Considering that this could be due to a modification of error distribution in relation to the original model introduced by linearization, a non-linear regression method was selected for obtaining the parameter values of the non-transformed equation. The initial values for the nonlinear solution were those obtained through the linear regression analysis. Fig. 3 and 4 show that the non-linear method had a better fitting than the linear one.

Values obtained for the Weibull equation parameters as a function of the length:diameter ratio, L/D, and superficial velocity, u_f, are given in Table 4. To develop a general model in terms of the operation variables: u_f and L/D, a quadratic model was employed to characterize the functionality of Weibull parameters; θ and b.

The final general expression obtained for these work operation conditions is given by:

$$\frac{C(t)}{Co} = \exp\,[(-t/E[\theta]^{E(b)}],$$

where the expected values $E(\theta)$ and $E(b)$ take the following form:

$$E(\theta) = -22.119 + 5.358\,(L/D) + 18.02\,(u_f) - 2.517\,(u_f)^2$$

$$E(b) = 3.954 + 0.525\,(L/D) + 0.0731\,(L/D)^2 - 0.132\,(u_f)^2 + 0.0391\,(L/D)\,(u_f)$$

In both cases multiple correlation coefficients exceed 0.99.

$C(t)/Co$ values obtained from the general equation based on parameter values presented in Table 4 are plotted in Fig. 3 and 4.

It can be demonstrated that fitting is adequate in the entire experimental $C(t)/Co$ range except in the last part of the curve where skin elutriation rate is higher than predicted by the model. This difference could be due to the degree of attrition reached towards the end of the operation, which favors skin separation to a larger extent than that proposed by the Weibull equation.

Drying

The effect of superficial velocity, length:diameter ratio and temperature on grape seed dehydration behavior is shown in Fig. 5, 6 and 7.

Table 3—Experimental runs

Experimental runs	Fluidization velocity (m/sec)	L/D Ratio	Temperature (°C)
Drying			
L/D Ratio effect	5.0	0.5, 1, 1.5, 2	25
Velocity effect	3.35, 5.0, 5.9	1	25
Temperature effect	5.0	1	25, 40, 50, 60
Separation			
L/D Ratio effect	5.0	1, 2, 3, 4	25
Velocity effect	3.35, 4.2, 5.0, 5.9	2	25

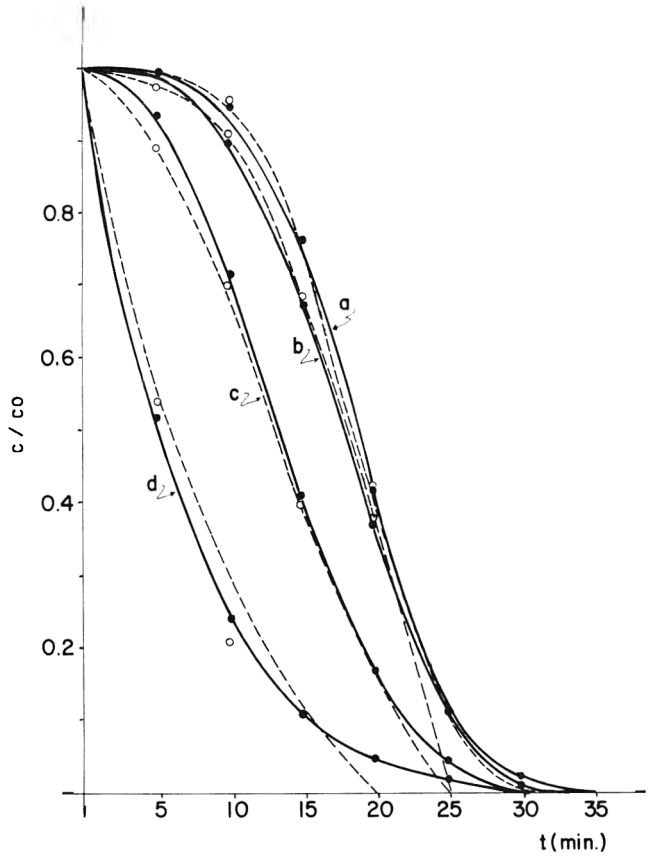

Fig. 3—Effect of the superficial velocity on grape pomace elutriation: (a) $u_t = 3.35$ m/sec; (b) $u_t = 4.20$ m/sec; (c) $u_t = 5.0$ m/sec; (d) $u_t = 5.9$ m/sec. (● predicted values, ○ experimental values).

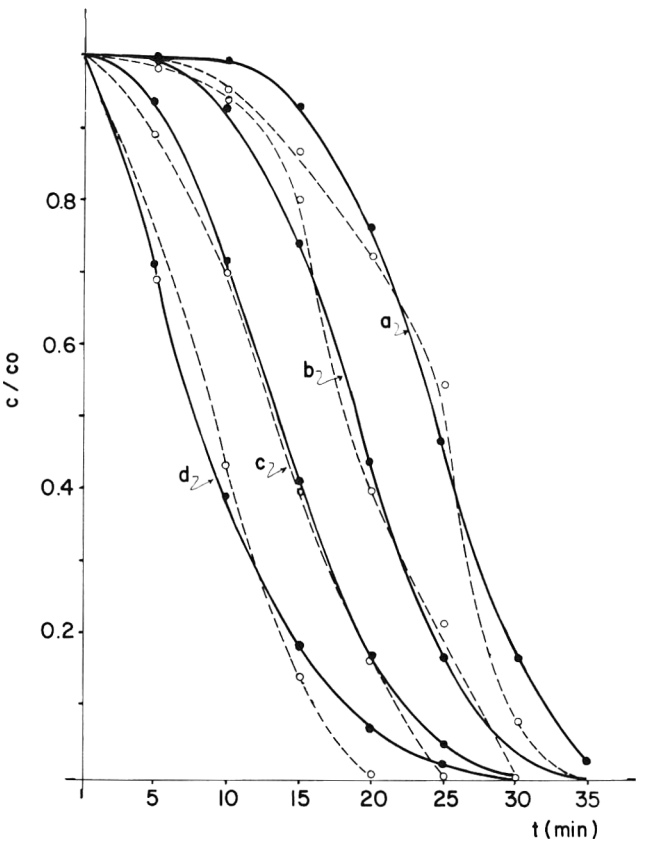

Fig. 4—Effect of the L/D ratio on grape pomace elutriation: (a) L/D = 4; (b) L/D = 3; (c) L/D = 2; (d) L/D = 1. (● predicted values, ○ experimental values).

It can be observed that drying curves showed two periods: a first one of constant drying rate in which the operation was controlled by the external resistance to mass transport and a second period of decreasing drying rate in which the operation was internally controlled.

Figure 6 illustrates that an increase in the bed length diminished the drying rate and thus the constant drying rate period has a more important role during the operation.

The contrary effect was observed when superficial velocity influence was analyzed (Fig. 5); higher air flows increased moisture elimination from solid surface, reducing the length of the constant drying rate period.

Figure 7 shows that from a certain temperature, drying curves could be analyzed solely in terms of a decreasing drying rate period. In other words, external mass transfer intensity reduces drying rate control to internal humidity movement in the solid.

Based on these results and considering the mathematical models proposed in the literature for each one of the drying periods (Kunii and Levenspiel, 1969), drying transport parameters were determined.

From experimental data during constant drying rate periods, correlations for external mass and heat transfer, between gas and solid-gas interface, were obtained by multiple linear regression. Expressions obtained are shown below:

$$S_h = 7.97 \times 10^4 (Re_p)^{1.92} \left(\frac{L}{D}\right)^{-0.58}$$

for $Sc = 0.458$; $85 \leq (Re_p) \leq 150$; $0.5 \leq (L/D) \leq 2.0$;

$$Nu = 8.23 \times 10^4 (Re_p)^{2.0} \left(\frac{L}{D}\right)^{-0.57}$$

for $Pr = 0.707$; $85 \leq (Re_p) \leq 150$; $0.5 \leq (L/D) \leq 2.0$, where, Re_p = particle Reynolds number, Sh = Sherwood number, Sc = Schmidt number, Pr = Prandtl number, and Nu =

Table 4—Weibull equation parameters as obtained by linear regression

	Effect of L/D ratio ($u_t = 5.0$ m/sec)			
Parameter	L/D = 1	L/D = 2	L/D = 3	L/D = 4
θ	10.299	15.586	21.399	26.275
b	1.497	2.210	3.577	4.701
	Effect of superficial velocity (L/D = 2)			
Parameter	$u_t = 3.34$ m/sec	$u_t = 4.2$ m/sec	$u_t = 5.0$ m/sec	$u_t = 5.9$ m/sec
θ	20.643	20.029	15.586	7.232
b	4.048	3.433	2.210	1.229

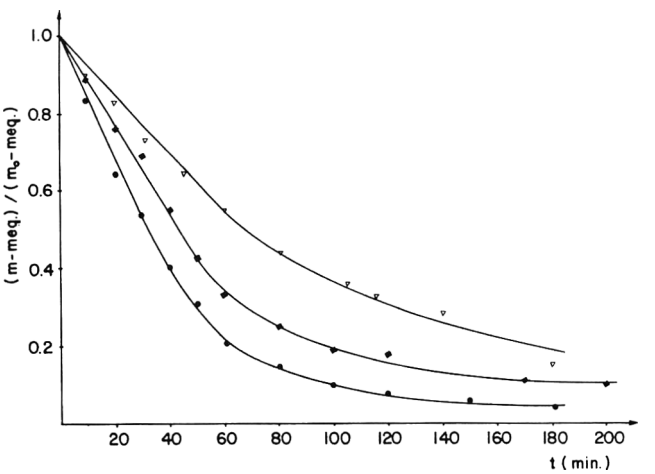

Fig. 5—Effect of air superficial velocity on drying time curves: ● 5.9 m/sec; ♦ 5.0 m/sec; ▽ 3.35 m/sec.

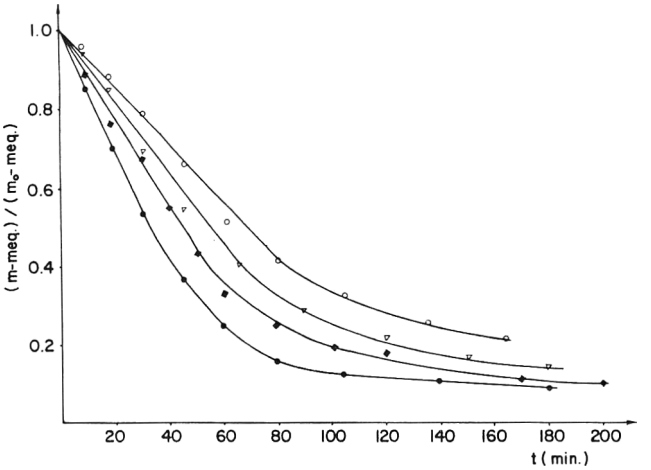

Fig. 6—Effect of the L/D ratio on drying time curves: ● L/D = 0.5; ♦ L/D = 1.0; ▽ L/D = 1.5; ○ L/D = 2.0

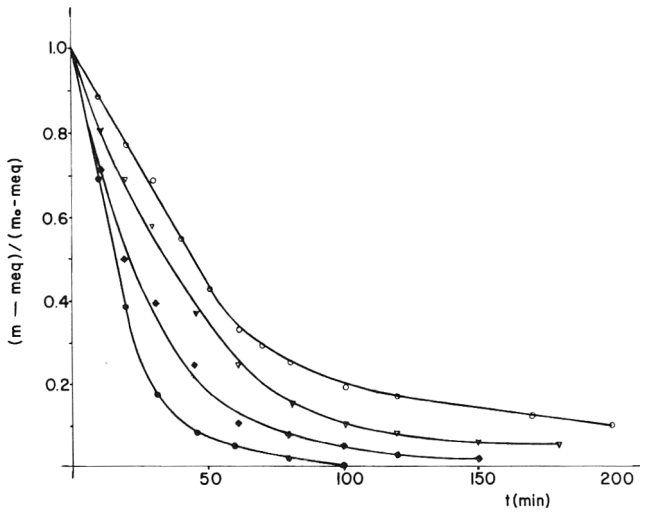

Fig. 7—Effect of temperature on drying time curves: ● T = 25°C; ♦ T = 40°C; ▽ T = 50°C; ○ T = 65°C.

Nusselt number. These dimensionless numbers are defined in terms of their specific variables in the nomenclature at the end of this paper. Exponents and coefficients of estimated correlations compare favorably with those reported in the literature for gas-solid experiences (Kuni and Levenspiel, 1969). On the other hand, it was observed that during the decreasing drying rate period, mass transfer Biot number was higher than 10, given the magnitude of the internal, D_L, and surface, kg, mass transfer coefficients. Therefore, it was concluded that the decreasing drying rate period is controlled by liquid molecular diffusion inside the solid (Crank, 1975).

Applying the solution to Fick's second law equation of molecular transport with constant boundary conditions, values of the diffusion coefficient were obtained. The order of magnitude of these coefficients, 10^{-10} m^2/sec were similar to those reported in the literature (Chirife, 1971). Molecular diffusion coefficient temperature dependence was explained by means of the Arrhenius equation:

$$D_L\ (m^2/sec) = 6.01 \times 10^{-6} \exp\ [-6969.35\ (cal/mol)/RT(°K)]$$

NOMENCLATURE

a_w = water activity
b = Weibull equation parameter
Co = initial bed skin constant
C(t) = bed skin content at any time
C_p = air specific heat
D = bed diameter
D_{AB} = air-water molecular diffusion coefficient
D_P = seed diameter
D_L = moisture molecular diffusion coefficient
E(θ) = θ expected value
E(b) = b expected value
F = constant in B.E.T. equation
G = air flow rate
h = external heat transfer coefficient
J = Henderson equation parameter
K = air thermal conductivity
Kg = surface mass transfer coefficient
Kg' = external mass transfer coefficient
L = bed length
m = moisture content
m_m = single layer moisture content
Nu = Nusselt number = h Dp/K
n = Henderson equation parameter
Pr = Prandtl number = Cpμ/K
R = gas constant
Re_p = particle Reynolds number = D_PG/μ
Sc = Schmidt number = $\mu/\rho\ D_{AB}$
Sn = Sherwood number = $kg'D_P/D_{AB}$
t = time
T = temperature
u_f = superficial velocity
u_{mf} = minimum superficial velocity

Greek letters

ρ = air density
μ = air viscosity
θ = Weibull equation parameter

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Influence of Sugars on Heat Inactivation, Injury and Repair of *Saccharomyces cerevisiae*

DANILA TORREGGIANI and R. T. TOLEDO

ABSTRACT

The type of sugar in the heating and recovery media affected the rate of inactivation and repair capability of a Chablis strain of *Saccharomyces cerevisiae*. The rate of heat inactivation decreased with decreasing a_w in glucose and fructose but not in sucrose solutions. At any a_w the order of susceptibility to inactivation of yeast cells was consistently: fructose, glucose, and sucrose. In fructose, a major proportion of the survivors exhibited sublethal injury. When suspended in solutions containing the various sugars after heating and incubated for up to 18 hr prior to plating, the type of sugar in the solution influenced the ability of cells to repair injury.

INTRODUCTION

OSMOTIC DEHYDRATION of fruits has received increasing attention in recent years as a gentle, energy efficient dewatering treatment to produce shelf stable fruits. A process consisting of osmotic dehydration of fruit pieces followed by packaging and mild heat treatment was recently proposed (Maltini and Torreggiani, 1981; Maltini et al. 1983). The mild heat treatment is done primarily to inactivate yeasts, which are the major spoilage organism in a vacuum-packaged product that has water activity (a_w) values of 0.94–0.96, pH of 3.5–4.0 and low redox potential.

Yeasts can grow at a relatively low a_w, high sugar concentration, and low pH (Leistner and Rodel, 1976). They also show increased heat resistance in dry foods (Corry, 1973). Gibson (1973) reported that heat resistance of two strains of osmophilic yeast increased in solutions of sucrose and in sucrose/glucose mixtures. Rose (1975) reported that several osmotolerant and nonosmotolerant yeast cells exhibited a decrease in cell volume and increased heat resistance in solutions of sucrose and sucrose/glucose mixtures but the same effect was not exhibited in polyethylene glycol. Beuchat (1981) showed that sucrose at levels up to 60% (a_w 0.892) protected five to six strains of yeast against heat inactivation. Juven et al. (1978) demonstrated that the resistance of yeast to inactivation in orange juice concentrate was higher than in acidified solutions containing mixtures of glucose, fructose, and sucrose in the same total sugar concentration as in the juice. Corry (1976a, b) showed that solute used in the heating medium had a profound effect in heat resistance of two strains of osmophilic yeast. Freeze-etch electron microscopy and turbidity (a measure of the degree of plasmolysis) indicated that solutes which caused the greatest increase in heat resistance plasmolysed the cells.

Besides increasing heat resistance of yeast cells, the heating medium also influence the extent of sublethal injuries and repair of thermally stressed cells. A review of injury and recovery of yeasts and molds was done by Stevenson and Graumlich (1978). Furthermore, Stevenson and Richards (1976) and Graumlich and Stevenson (1978) showed that delayed plating of thermally stressed cells of *Saccharomyces cerevisiae* after storage at 22°C resulted in increased counts, primarily due to repair of thermal injury.

This study was carried out to investigate the effect of a_w, different sugars and mixtures of sugars on the heat resistance and thermal injury repair capability of a Chablis strain of *Saccharomyces cerevisiae*. The information generated should be useful in defining effective heat treatments for stabilizing sweetened fruit products for storage at ambient temperatures.

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Table 1—Composition of the sugar solutions in which the Saccharomyces cerevisiae *strain was heated*

aw	Sucrose (g)	Glucose (g)	Fructose (g)	H_2O (g)
0.87	65.0	—	—	35.0
0.87	—	55.0	—	45.0
0.87	—	—	55.0	45.0
0.90	59.5	—	—	40.5
0.90	—	48.0	—	52.0
0.90	—	—	48.0	52.0
0.95	45.0	—	—	55.0
0.95	—	32.0	—	68.0
0.95	—	—	32.0	68.0
0.95	19.7	—	18.5	61.8
0.95	6.5	—	28.0	65.5
0.95	36.5	—	6.0	57.5

MATERIALS & METHODS

Organisms and cultural conditions

A Chablis wine strain of *S. cerevisiae* Hansen, obtained from stock cultures at the Univ. of Georgia, Food Science Dept. was used. Stock cultures were maintained on potato dextrose agar (PDA, pH 5.5) slants at 4°C. The yeast cells were cultured in YMPG broth (Yeast extract, malt extract, peptone, glucose at 3, 3, 5, and 10g L^{-1}, respectively, in deionized water, pH 5.5). Cultures were inoculated into 100 mL of sterile YMPG broth in 250 mL Erlenmeyer flasks and incubated for 44–48 hr on a rotary shaker (150 rev/min) at 25°C.

Preparation of heating media

Solutions of analytical grade sucrose, glucose and fructose in deionized water were prepared and autoclaved at 121°C for 15 min. The pH of all sterilized solutions was adjusted to 3.5 by adding sterile 1N H_3PO_3. Five milliliters of 40% sterile Bacto-peptone (Difco) solution was then added to 95 mL of the pH adjusted sugar solution to give a final concentration of 2% peptone to serve as a buffering agent. Yeast cells were heated in solutions containing one of three different sugars at water activities of 0.87, 0.90 and 0.95 or in solutions containing various proportions of sucrose and fructose. Table 1 shows the composition of the heating solutions. The amount of sugars needed to give the desired a_w in the solutions was formulated according to Norrish's (1966) formula and the a_w values of the prepared solutions were then measured at 25°C using a Beckman hygroline instrument equipped with a NOVOSINA model EBS sensor and sample dish assembly (Beckman Inst., Cedar Grove, NJ). The instrument readings were regularly calibrated using salt mixtures provided with the instrument. Measured and formulated a_w values were the same.

Heat resistance tests

Cells of *S. cerevisiae* were subjected to sublethal thermal stress utilizing the procedure described by Beuchat (1982). One milliliter of vegetative cells suspension was added to 250 mL Erlenmeyer flasks

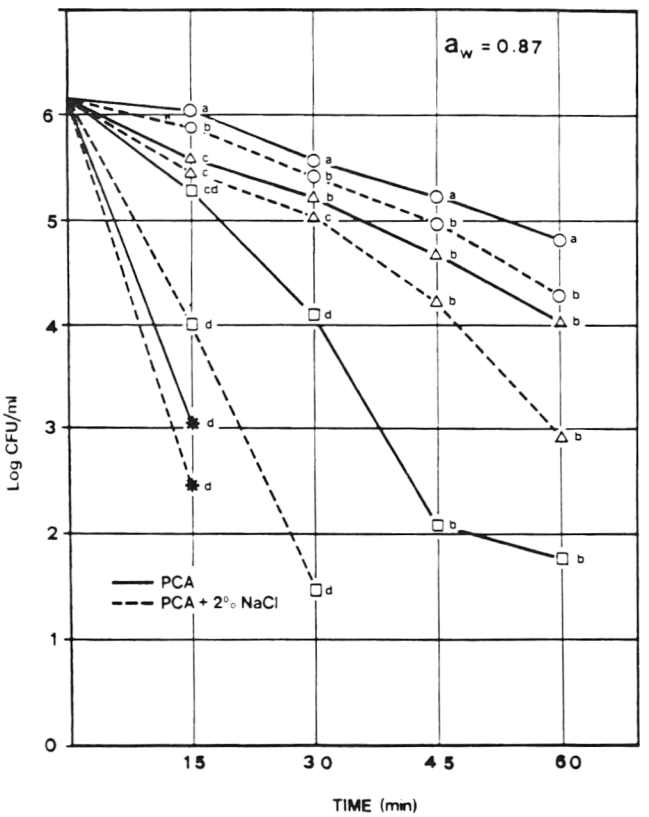

Fig. 1—Thermal inactivation curves for S. cerevisiae *at 50°C in 2% Bacto-Peptone solutions containing ○ sucrose, △ glucose, and □ fructose at pH 3.5, a_w 0.87. * represents control. Absence of data point at a particular heating time indicates complete inactivation. The same letter over data points indicates no significant difference ($P \leq 0.05$) in the data of that time of heating.*

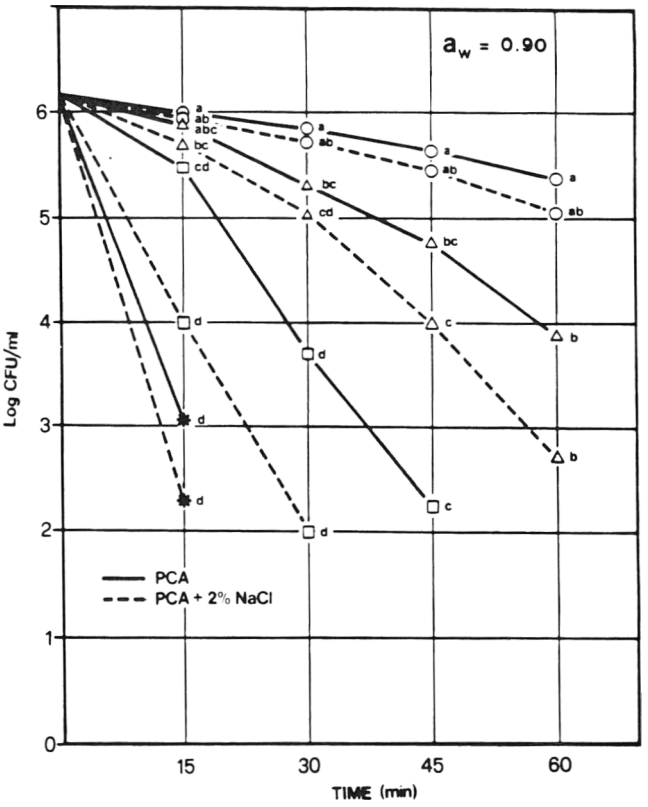

Fig. 2—Thermal inactivation curves for S. cerevisiae *at 50°C in 2% Bacto-Peptone solutions containing ○ sucrose, △ glucose, and □ fructose at pH 3.5, a_w 0.90. * represents control. Absence of data point at a particular heating time indicates complete inactivation. The same letter over data point indicates no significant difference ($P \leq 0.05$) in the data of that time of heating.*

containing 100 mL of heating medium (pH 3.5) which had been tempered to 50°C in a gyratory water bath shaker and maintained under constant agitation (125 rpm). Samples were withdrawn after 15, 30, 45, and 60 min heating. Heated and nonheated cells were diluted in 0.1M potassium phosphate buffer (pH 7.0) containing 20% (w/v) of sucrose; 0.1 mL samples were surface plated on either PCA (Plate Count Agar - Difco Laboratories, Detroit, MI) or PCA + 2% sodium chloride (pH 7.0). Addition of 2% NaCl to the PCA prevented the recovery of injured cells (Tsuchido et al., 1972; Shibasaki and Tsuchido, 1973; Beuchat, 1982) and the difference in colony forming units between the two media gave a measure of the number of injured cells. Sodium chloride was added to the PCA before it was autoclaved (121°C for 15 min). Since the inherent heat resistance of organisms varies from culture to culture, each replicate which included the full range of sugar solutions was done on the same day using the same suspension of organisms.

Repair of heat-injured cells

The effect of storage in different sugar solutions at the same a_w on the repair of heat-stressed cells of *S. cerevisiae* was evaluated. Cells of *S. cerevisiae* thermally stressed at 50°C for 45 min in solutions of different sugars at the same a_w were plated in PCA and PCA + 2% NaCl immediately after heating, and after the heated cells were suspended in solutions with the same composition as the heating media and incubated at 22°C up to 18 hr. Samples were withdrawn at 6 hr intervals during incubation and plated. Nonstressed cells were treated in a similar manner to serve as a control.

Statistical analyses

Data were presented as means of a minimum of three replications run in duplicate. The number of survivors at any given time of heating at a given water activity was statistically analyzed using the analysis of variance procedure to determine differences in the effect of the types of sugars in the heating medium. A least significant difference was then computed and used to determine differences between individual means.

RESULTS & DISCUSSION

Heat resistance of yeast cells in solutions of different sugars

Figures 1 to 3 show the inactivation curves for the Chablis strain of *S. cerevisiae* at 50°C in aqueous solutions of 2% Bacto-peptone (control) and the same solution containing different sugars at different a_w. The sugars protect yeast cells against heat injury as shown by the most rapid inactivation in the control compared to the sugar solutions. Sucrose gave the most protection against heat injury of the cells while fructose offered very little protection. The number of cells that survived heating in fructose approached those heated in the control solution. The number of survivors at any heating time at any water activity was lowest in fructose and highest in sucrose. The degree of protection provided by sucrose was most pronounced at a_w 0.90 (Fig. 2) where the highest number survived and among the survivors the least number of cells were inhibited by the presence of NaCl in the recovery media. The degree of protection offered by glucose against cell inactivation was consistently between that of sucrose and fructose. At a_w 0.95 (Fig. 3) a larger proportion of the survivors exhibited sublethal injury as indicated by the highest differences in the number of cells recovered in PCA and those recovered in PCA + 2% NaCl, compared to the lower a_w. The data also showed that glucose and fructose both exhibited increasing protection against inactivation of cells with decreasing water activity but sucrose allowed more susceptibility to inactivation at a_w 0.87 (Fig. 1) compared to a_w 0.90 and 0.95.

The influence of a_w and the type of sugars on heat inactivation of yeast cells can be readily seen in a plot of survivors and the percentage exhibiting metabolic injury after 30 min of heating (Fig. 4). Most (98–99%) of the survivors after heating in fructose solution had sublethal injury, and this level of injury

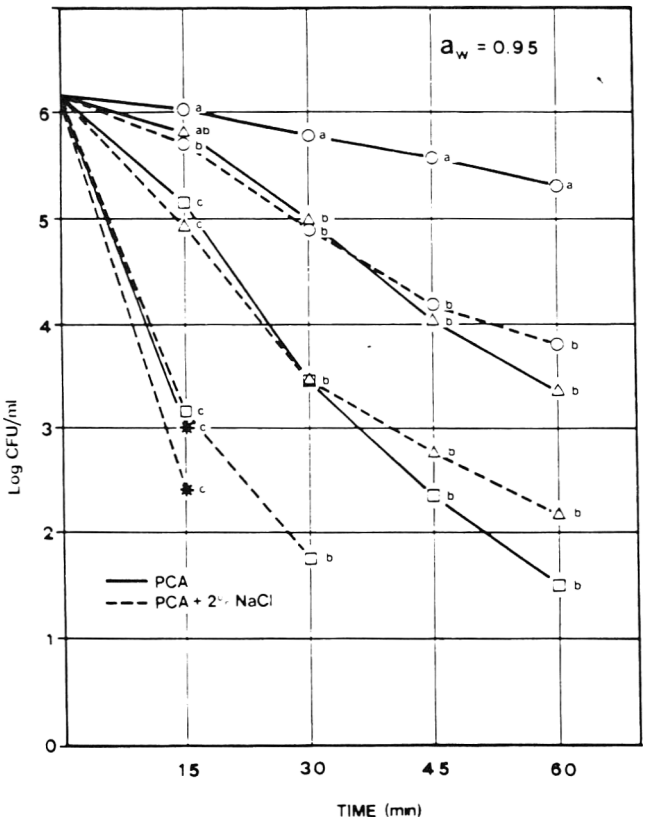

Fig. 3—Thermal inactivation curves for S. cerevisiae *at 50°C in 2% Bacto-Peptone solutions containing ○ sucrose, △ glucose, and □ fructose at pH 3.5, a_w 0.95. * represents control. Absence of data point at particular heating time indicates complete inactivation. The same letter over data point indicates no significant difference ($P \leq 0.05$) in the data of that time of heating.*

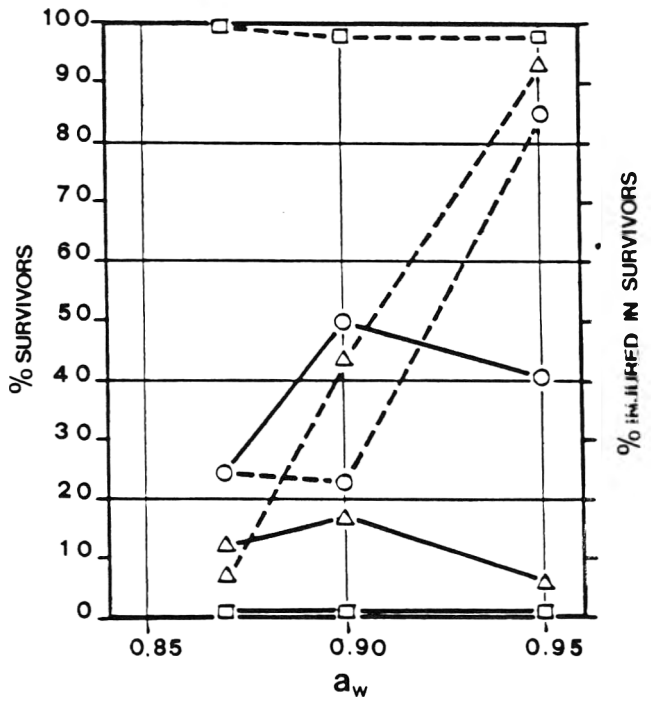

Fig. 4—Percentage of survivors (_____) and percentage of injured cells of S. cerevisiae *in survivors (- - - - - - -) after 30 min at 50°C as a function of a_w. ○ sucrose, △ glucose, □ fructose (composition in Table 1).*

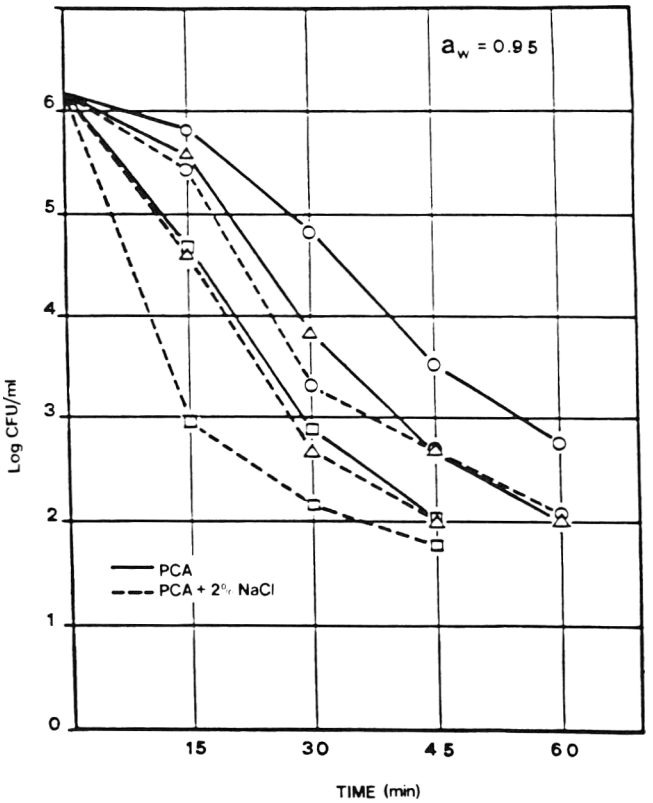

Fig. 5—Thermal inactivation curves for S. cerevisiae *at 50°C in 2% Bacto-Peptone solutions with mixtures of: ○ 36.5% sucrose and 6.0% fructose, △ 19.7% sucrose and 18.5% fructose, □ 6.5% sucrose and 28.0% fructose, w/w (pH 3.5). Lack of data at particular heating time indicates complete inactivation.*

was the same at a_w from 0.87–0.95. The increase in protection with decreasing a_w of glucose solutions was evident in a steady decrease of sublethal injury among the survivors as a_w decreased. With sucrose on the other hand, there was a dramatic drop in cells with sublethal injury between a_w 0.95 and 0.90, but the percentage of survivors with sublethal injury did not change between a_w 0.90 and 0.87. The number of cells surviving the 30 min treatment at 50°C showed an increasing trend with decreasing a_w when the heating medium contained either glucose or fructose, whereas, a significantly reduced number survived at a_w 0.87 compared to 0.90 when sucrose was present.

The difference in the influence of the various sugars on thermal injury of yeast cells appeared to be due to the chemical nature of the compound rather than the physical characteristics of the heating solutions. Since solutions containing the three sugars were each formulated with the same water activity, the osmotic pressure of the solutions should be identical. The influence of viscosity of the heating medium was minimized because of the agitation that was imparted during heating. Furthermore, solutions containing glucose and fructose have exactly the same sugar concentrations and the same viscosity but thermal inactivation proceeded rapidly in fructose compared to glucose solution. The protection offered by an increased viscosity was also not apparent in the case of sucrose where higher susceptibility to inactivation by heat was exhibited at a_w 0.87 compared to a_w 0.95.

Corry (1976a) found that heat resistance of *S. rouxii* in solutions of glucose, fructose, sucrose, glycerol, and sorbitol was highest in sucrose and sorbitol; these two sugars also caused plasmolysis. This effect was not as marked in the other solutions presumably because the solutes were able to permeate through the cytoplasmic membrane. This theory, however, does not explain why there is little change in heat resistance with increasing fructose concentration or why more cells survived in sucrose at a_w 0.95 compared to a_w 0.87 (Fig. 1 and 3). The data suggested that the type of solute surrounding the cell af-

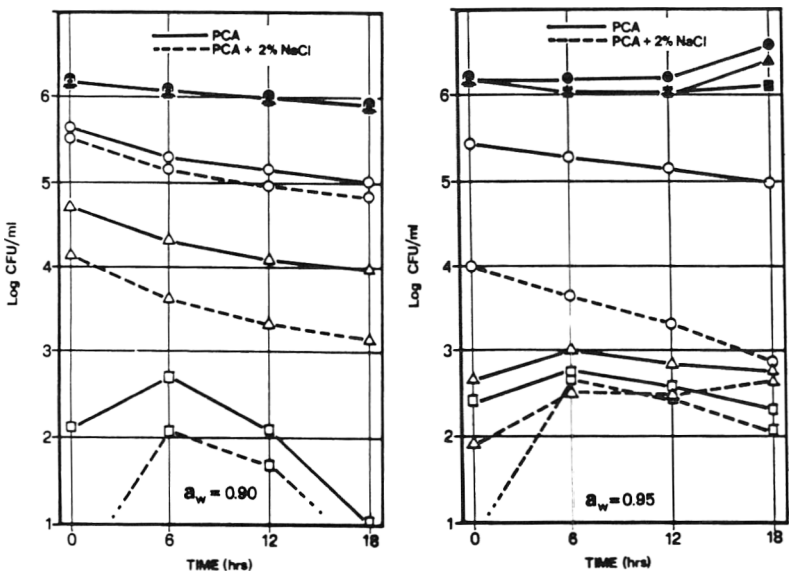

Fig. 6—Effect of storage at 22°C in 2% Bacto-Peptone solutions with different sugars (pH 3.5) on the apparent number of survivors of S. cerevisiae *cells treated for 45 min at 50°C. ○ sucrose, △ glucose, □ fructose (composition in Table 1) ●, ▲, ■, control.*

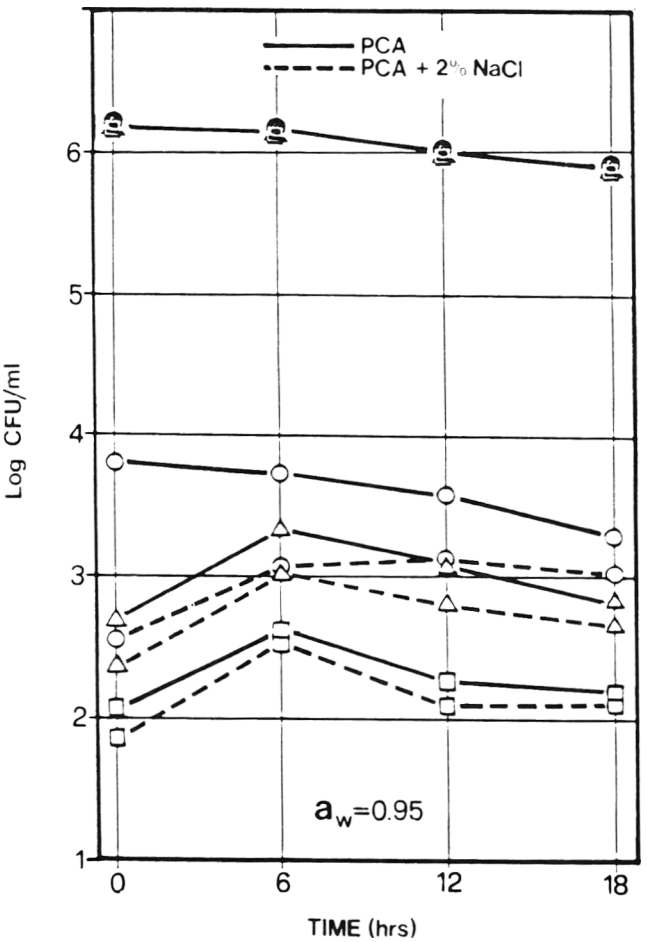

Fig. 7—Effect of storage at 22°C in 2% Bacto-Peptone solutions with different sugar mixtures on the apparent number of survivors of S. cerevisiae *cells treated for 45 min at 50°C (pH 3.5). ○ 36.5% sucrose and 6.0% fructose, △ 19.7% sucrose and 18.5% fructose, □ 6.5% sucrose and 28.0% fructose, w/w. ●, ▲, ■ control.*

fected the ability of the cell to repair heat injury. On the other hand, yeast has also been shown to leak nucleotidic material into surrounding media containing glucose (Lee and Lewis, 1968). Hagler and Lewis (1974) demonstrated that yeast cells heat shocked in a medium containing utilizable sugar exhibited characteristics of organisms with ruptured cytoplasmic membranes. Thus, the influence of sugars in the suspending medium on leakage of cell contents may be related to the mechanism of active transport of nutrients across cell membranes, a mechanism which also increases the vulnerability of the cells to heat injury.

The influence of a_w on the inactivation rate was minimal in fructose (Fig. 4), and the inactivation curve approached that of fructose when a 1:1 mixture of fructose and sucrose was present in the heating medium (Fig. 5). The protection offered by the presence of sucrose is evident in Fig. 5 where the percentage of cells with sublethal injury appear to be lower in the mixture compared to that in fructose alone.

Repair of heat injured cells

The data presented in Fig. 6 show the effect of holding the cells in a solution having the same composition as the heating medium at 22°C on repair of cells of *S. cerevisiae* thermally stressed at 50°C for 45 min in solutions (a_w 0.95 and 0.90) of different sugars. At a_w 0.90 nonheated cells stored for 18 hr in aqueous solutions of sucrose, glucose and fructose with 2% of Bacto-peptone showed no growth and no difference in recovery between PCA and PCA + 2% NaCl. On the other hand differences between recovery of heat-stressed cells on PCA and PCA + 2% NaCl were quite apparent when samples were plated immediately after heating. After 18 hr of storage in sucrose and glucose the percentage of cells with sublethal injury was not reduced and the plate counts on both media decreased constantly from 0 to 18 hr of storage. On the contrary, after 6 hr of storage in fructose, plate counts in both PCA and PCA + 2% NaCl increased and the percentage of cells with impaired ability to reproduce in the presence of NaCl was reduced considerably. From 6 to 18 hr of storage in fructose the number of colony forming units (CFU) in both recovery media showed a marked decrease.

At a_w 0.95 nonheated cells stored for 18 hr in sucrose and glucose showed an increase in number of CFUs, but no increase was observed in fructose. It has been previously pointed out that at a_w 0.90 and 0.95 differences between recovery of heat stressed cells on PCA and PCA + 2% NaCl were quite apparent when samples were plated immediately. After 18 hr of storage in sucrose the proportion of cells with sublethal injury was not reduced. On the contrary, after 6 hr of storage in glucose and in fructose, plate counts in both media increased and especially for fructose, the difference in CFU on the two media was reduced considerably. These data indicate that the

different sugars influenced the capability of the cells to repair heat-induced sublethal injury differently.

The data presented in Fig. 7 shows the effect of storage in the heating medium containing sugar mixtures at 22°C of cells of *S. cerevisiae* thermally stressed at 50°C for 45 min in three different sucrose-fructose mixtures at a_w 0.95. Nonheated cells stored for 18 hr showed no growth and no difference in CFU in PCA and PCA + 2% NaCl. As for the individual sugars solutions, the thermal stress at 50°C resulted in reduced colony forming ability on PCA + 2% NaCl as compared with PCA, when samples were plated immediately. After 6 hr of storage in all the three different mixtures, plate counts in both PCA and PCA + 2% NaCl increased, and the difference in recovery on the two media was partially reduced.

CONCLUSIONS

The type of sugar present in the heating medium influenced the rate of heat inactivation of yeast cells and the extent of sublethal injury on the survivors. Fructose allowed the most rapid inactivation rate and a major proportion of the survivors exhibited impaired ability to form colonies in the presence of 2% NaCl in the recovery media. Sucrose gave the most protection and increased the heat resistance of the cells. The heat resistance of cells in fructose, glucose, and sucrose increased with decreasing water activity except for sucrose at a_w 0.87. The influence of the different sugars on heat resistance appeared to be due primarily to their ability to affect the metabolic activity of the injured cell, as opposed to a purely physical effect of viscosity, diffusivity, or osmotic pressure of the solutions. The results of this study indicated that when preparing products with reduced water activity by treatment with sugar solutions followed by a mild heat treatment, the use of fructose rather than sucrose would reduce the severity of the required heat treatment and minimize quality changes in the product that might result from the heating process.

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Origin of *N*-Nitrosomorpholine Contamination in Margarine

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ABSTRACT

Previous studies have indicated that certain margarine samples may contain traces (~ 1 ppb) of NMOR. The results of this present study indicated that packaging papers were the source of the contamination. Waxed wrappings and margarine samples taken from 1 cm outer layer of margarine blocks were found to contain NMOR, but those taken from the center of the margarine blocks were always negative. This suggests migration of NMOR from wrappings to the outer layers of the margarine blocks. Samples packaged in aluminum-backed papers, specially coated waxed papers, or plastic containers were negative as determined by Thermal Energy Analyzer technique.

INTRODUCTION

THERE ARE conflicting reports in the literature as to the occurrence of volatile nitrosamines in cooking oils and margarines. Hedler et al. (1979) reported the presence of 1–28 ppb *N*-nitrosodimethylamine (NDMA) and/or *N*-nitrosodiethylamine (NDEA) in 24 out of 61 samples of various cooking oils and slightly lower levels (1–7 ppb) of the same nitrosamines in 37 out of 107 margarines analyzed. But in a later study, Sen and Seaman (1981) were unable to substantiate the above findings for cooking oils. They observed that various cooking oils available for sale to consumers in Canada were free (detection limit 0.1–0.2 ppb) of volatile nitrosamines but 4 out of 14 margarines analyzed contained traces (1.7–3.8 ppb) of *N*-nitrosomorpholine (NMOR). One of the possible sources of the NMOR contamination in margarine was thought to be morpholine, an amine, often used as a boiler water additive in food processing plants. In a follow-up study, however, Sen and Seaman (1981) were unable to conclusively determine the source of NMOR.

In another study from Germany, Aitzetmueller and Thiele (1982) analyzed 20 samples of various margarines procured from four different countries; all gave negative (<0.5 ppb) results. In the preliminary stage of their work, however, these workers detected extremely low levels (0.5–2 ppb) of NMOR in some margarines, but after a much more thorough investigation these researchers discovered that the NMOR detected in the margarines originated from contaminated dichloromethane used in the analysis. When they repeated the analyses with purified (redistilled) dichloromethane, the same samples gave negative results. Therefore, they concluded that margarines as such were free of NMOR or any other volatile nitrosamines. Fiddler et al. (1981) detected only traces (0.2–1 ppb) of NDMA in several cooking oils and margarines marketed in the U.S.A.

Although the use of contaminated dichloromethane in the analysis of margarine (or other foods) for nitrosamines could lead to false-positive results, it is highly unlikely that this was the case with our previous studies (Sen and Seaman, 1981). In these studies (Sen and Seaman, 1981), each bottle of commercially available dichloromethane was redistilled and tested for nitrosamine contamination before use; moreover, the rest of the samples in the same survey were analyzed using the same dichloromethane but were found to be negative (<0.5 ppb for NMOR). Therefore, the possibility of contamination from dichloromethane was completely ruled out.

A more likely explanation for the source of NMOR in margarine came from a study by Hoffmann et al. (1982). While analyzing snuff for nitrosamines, these workers discovered that NMOR in snuff originated from waxes used as coating on snuff containers. They also detected traces (0.9–3.3 ppb) of NMOR and morpholine in one sample each of butter, cream cheese, yogurt, cottage cheese, frozen vegetables and four samples of semi-soft cheeses. The respective containers also contained traces of NMOR and much higher (35–17,200 ppb) levels of morpholine. Since margarines are also packaged in waxed papers, it seemed likely that waxes might also have been the source of NMOR in margarines. Consequently, a study was undertaken to investigate this possibility; the results are reported herein. It should be noted that Hotchkiss and Vecchio (1983) have also detected traces of NMOR and morpholine in some paper and paperboard direct contact food packagings.

MATERIALS & METHODS

Samples

All the margarine samples used in this study were purchased in the retail outlets in Ottawa, Ontario. Samples packaged in a variety of containers (waxed papers, aluminum-backed papers, plastic tubs) were used.

Analysis for volatile nitrosamines

A 20-g aliquot of the sample was analyzed by a low temperature vacuum distillation method from 3N KOH as described previously (Sen et al., 1979). The end determination was carried out by gas-liquid chromatography – Thermal Energy Analyzer (GLC-TEA) technique (Fine and Rounbehler, 1975). To routinely monitor the efficiency of the method, each sample was analyzed after spiking with 10 ppb of *N*-nitrosodi-*n*-propylamine as an internal standard. The percentage recoveries of the internal standard as well as that of the other volatile nitrosamines (NDMA, NDEA, NMOR) were all highly satisfactory (80–95%). The lower detection limit of the overall method was 0.1–0.2 ppb for NDMA and NDEA and approximately 0.5 ppb for NMOR.

As in the previous studies (Sen et al., 1979; Sen and Seaman, 1981) each bottle of dichloromethane was redistilled from an all-glass apparatus, and the purified solvent was tested (400 mL concentrated to 1.00 mL) for nitrosamine contamination before use. A complete reagent blank was also carried out with each batch of other reagents.

For packaging papers the entire paper from a single block of margarine was cut into small pieces and analyzed as above for volatile nitrosamines. The method was modified slightly for aluminum-backed papers because of vigorous reaction of aluminum with alkali and formation of hydrogen. In these cases, the sample was first extracted with dichloromethane (by soaking and occasional stirring in a beaker) and then the extract was distilled from 3N KOH as described previously (Sen et al., 1979).

Experimental design

To determine whether NMOR in margarine was originating from packaging paper, all samples packaged in various papers were subdivided into three parts as outlined below and each part was separately

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Table 1—NMOR detected in margarine and packaging materials

Brand[a]	Type of packaging	NMOR detected: Margarine, inner core NMOR, ppb	NMOR detected: Margarine, outer layer NMOR, ppb	NMOR detected: Packaging materials, NMOR, ng per package
A_1	Waxed paper	N[b]	1.4	24.7
A_2	Waxed paper	N	N	34
B	Waxed paper	N	0.5	6.7
C_1	Specially coated waxed paper	N	N	N
C_2	Specially coated waxed paper	N	N	62
C_3	Specially coated waxed paper	N	N	72.7
C_4	Specially coated waxed paper	N	N	18.8
D_1	Waxed paper	N	N	31.7
D_2	Waxed paper	N	1.1	5
E	Aluminum-backed paper	–	N	N
F	Aluminum-backed paper	–	N	N
G	Paper (unknown type)	–	N	N
H	Paper (unknown type)	N	N	N

[a] Different subscripts indicate samples from different lots of the same brand.
[b] N = none detected (<0.5 ppb).

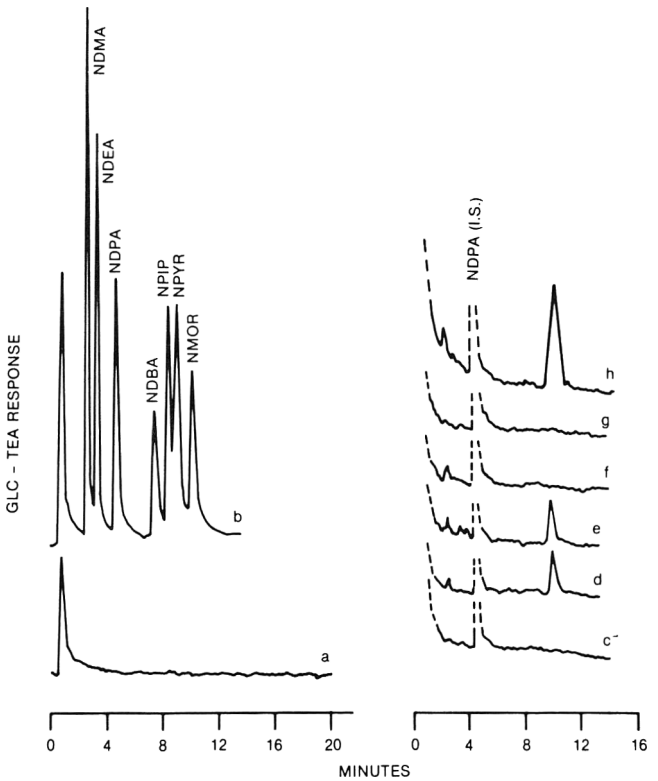

Fig. 1—Typical gas chromatograms: (a) concentrated dichloromethane blank, (b) approximately 500 pico grams of each of volatile nitrosamine standards (NDBA = N-nitrosodi-n-butylamine; NPIP = N-nitrosopiperidine; NPYR = N-nitrosopyrrolidine); (c) sample from center of margarine block, brand D_2; (d) sample from outer layer of above margarine; (e) wrapping from above margarine; (f) sample from center of margarine block, brand C_3; (g) sample from outer layer of same margarine block; and (h) wrapping from same margarine (brand C_3) block. See text for details about the two packaging papers. GLC conditions: 2.75m × 3.2 mm (o.d.) nickel tubing packed with 10% Carbowax 20M and 5% KOH on 100-120 mesh Chromosorb WHP. Injection port and transfer line (from column to TEA) temperatures were 200°C and 290°C, respectively. GLC-TEA analysis was carried out under temperature programming from 140°C to 170°C at the rate of 49°C/min. TEA attenuation at 4. For TEA operating conditions see Sen et al. (1979) and Fine and Rounbehler (1975).

analyzed for its nitrosamine content: (a) The entire packaging paper cut into small pieces; (b) a 20-g aliquot of margarine cut out from 1 cm outer layer of the block; and (c) a 20-g aliquot carefully taken from the center of the block. The data were then evaluated to determine if there were any relationships between NMOR levels in the packaging papers and those in the outer and inner layers of the margarine blocks.

RESULTS & DISCUSSION

THE DATA (Table 1) indicate that many of the waxed paper packings contained 5–73 ng NMOR per package. Initially, we were puzzled by the fact that three out of four of the packaging papers from brand C margarines contained significant amounts (up to 73 ng) of NMOR but none could be detected in the margarines. On close examination of the wrappers, it became apparent that they were a special type of waxed paper; the inner (that facing the margarine) surface was coated with a shiny plastic spray and the outer surface was coated with wax. The plastic coating most likely prevented migration of NMOR into the margarine. In the other cases (brands A, B and D), which were wrapped with normal waxed papers, some samples taken from the outer layer of the margarine blocks contained minute traces of NMOR. This suggested migration of NMOR from the waxed paper to the outer layer of margarine samples analyzed. None of the samples taken from the inner layers contained any detectable level of NMOR. Although it is not clear exactly how the packaging papers got contaminated with NMOR, it is highly likely that NMOR could have originated or formed from morpholine which is a known contaminant in packaging papers (Hotchkiss and Vecchio, 1983; Hoffman et al., 1982).

In addition, six samples of margarines packaged in plastic tubs (small or medium size) and two samples of cut-out pieces of plastic containers from the above tubs were analyzed. All six margarines as well as the two plastic containers were found to be negative. These samples consisted of one each of brands C and G and four other brands not mentioned in Table 1. Therefore, it was concluded that margarines as such are free of nitrosamines, and NMOR occasionally detected in margarines originates from waxed-paper packagings.

In view of the above findings, it seemed highly unlikely that the few positive findings of NMOR in margarines, as reported here and previously (Sen and Seaman, 1981), were due to the use of NMOR-contaminated dichloromethane used for the analysis of the samples. Figure 1 illustrates typical chromatograms obtained during the GLC-TEA analysis of various samples.

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Frying Oil Deterioration and Vitamin Loss During Foodservice Operation

B. L. CARLSON and M. H. TABACCHI

ABSTRACT

Oxidation and vitamin E loss in four frying oils (two partially hydrogenated soybean oils, one with methyl silicone, the other with tertiary butyl hydroquinone, citric acid and dimethyl siloxane added; a semi-solid hydrogenated soybean and palm oil shortening with mono and diglycerides added; and 100% corn oil) were studied under experimental and 'actual' operational conditions. Vitamin E loss in the frying oil increased significantly with increasing fatty acid oxidation. Added antioxidants, vitamin E and hydrogenation of fat decreased the rate of vitamin E loss. No significant change in vitamin E of the French fries occurred during 4 days of commercial frying; a significant increase in French fry fat uptake improved the 40% reduction in vitamin E of the frying oil. Vitamin C in the French fries (a major source of the vitamin in fast food meals) decreased significantly as the vitamin E content of the oils was reduced.

INTRODUCTION

NUTRIENT RETENTION during foodservice preparation of foods has become more important as consumers eat more meals away from home. The nutrient content of these meals depends on both the nutritional content of the products selected and the treatment of these products during preparation. The effect of both product characteristics and typical foodservice preparation methods on nutrient retention must be determined to enhance the nutrient content of restaurant meals. In addition, recent proliferation of foodservice products, preparation methods and emphasis on nutritional promotion suggest the need for evaluation of preparation losses and the final nutritional content of menu items at the point of sale to the consumer (Anon., 1980a, b, 1983; LaChance, 1973, 1975; Niepold, 1983).

Foodservice nutrient retention research contributes most to product development and best meets the information needs of the foodservice operator when designed according to the guidelines set forth by Livingston and Chang (1979). Dietary vitamin E is of nutritional concern with its currently advocated increase concomitant with an increase in polyunsaturated fatty acids (PUFA); although most foods high in PUFA are also high in vitamin E, this is not true in foodservice operations (Bunnel et al., 1965). Vegetable oils, used in the preparation of French fries, are one of the richest sources of vitamin E in restaurant meals, yet they vary considerably in α-tocopherol (0-125 mg/100g) (IFT Scientific Status Summary, 1979; Herting and Drury, 1963; Hunter, 1981). French fries provide the major source of both vitamins E and C in fast food meals (Bunch, 1984; Pao and Mickle, 1981). Retention of vitamin C in French fries is important as vitamin C is often low in meals consumed away from home and is a nutrient of concern for some U.S. population segments. Further, losses of vitamin E and C should be considered since they may be extensive in deep fat frying and frozen storage of fried foods (Landers and Rathmann, 1981; Karel, 1979; Osborne and Voogt, 1978; Livingston et al., 1973). Frying is one of the most common food preparation methods throughout the foodservice industry, and French fries are one of the best selling menu items at every menu price level in the restaurant industry (Anon, 1982a, b).

The objectives of this study were: (a) to quantitate α-tocopherol loss in four different frying oils as a function of time of heating at a typical frying temperature and in relation to the oxidation occurring in the fat; (2) to study the relationship between α-, γ-tocopherol losses occurring in frying oil and tocopherol and vitamin C of French fries over a 4-day period of commercial frying.

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MATERIALS & METHODS

Experimental heat treatment of frying oils under laboratory conditions

The effect of prolonged heating on fatty acid oxidation and loss of α-tocopherol in frying oils was studied by heating four commercial frying oils in Hotpoint 7.6L (2 gal) capacity fryolators (Model HK3) at 177°C (350°F). The oils were: (1) a partially hydrogenated soybean oil with methyl silicone added; (2) a partially hydrogenated soybean oil with tertiary butyl hydroquinone (TBHQ), citric acid and dimethyl siloxane added; (3) a semi-solid hydrogenated soybean and palm oil shortening and mono- and diglycerides added; and (4) 100% corn oil. The oils were sampled for analysis at six different times before and during heating at 177°C (350°F) (Table 1).

Frying oil used in actual foodservice operations

The second experiment was designed to study the α, γ-tocopherols, vitamin C, fat and moisture content of French fries. This study was also repeated on two separate occasions. French fries were prepared in frying oil at a temperature of 177°C (350°F) in a 15.2L (4 gal) capacity fryolator (Model F-108). The amount of product prepared and the addition of replacement oil was monitored during 4 consecutive days of production (Table 2). The frying oil (a partially hydrogenated soybean oil with TBHQ, citric acid and dimethyl siloxane added) and french fries (frozen, commercial 2.3 kg (5 lb) foodservice pack) were sampled for analysis prior to preparation. Samples of the oil and fries were taken at the beginning and end of foodservice operation on day 1 and at the end of foodservice operation of the next 3 consecutive days.

Methods of analysis

Peroxide values, free fatty acids, vitamin C (reduced ascorbic acid) and proximate analysis of moisture and fat were determined in duplicate according to AOAC (1975).

Tocopherol was determined by reverse phase high pressure liquid chromatography (HPLC) on a Beckman 110A HPLC unit following Folch extraction of French fries (Folch and Lee, 1957), saponification and reconstitution of the samples in absolute ethanol. The saponification procedure used was as follows: An aliquot of 200 mg of extract lipid was placed in a 20 mL screw cap tube. A volume of 1 mL 95% ethanol containing 0.02% each butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), 5.0% propyl gallate and 3.0 mL 5% KOH in ethanol were added. The tubes were flushed with N_2, capped and mixed on a vortex. Next the tubes were heated 10 min at 60–65°C and cooled under tap water. The tubes were flushed with N_2, capped and inverted to mix the phases. The hexane phase was removed and the hexane extraction repeated with partially hydrogenated soybean oil with TBHQ, citric acid and dimethyl siloxane added) and french fries (frozen, commercial 2.3 kg (5 lb) foodservice pack) were sampled for analysis prior to preparation. Samples of the oil and fries were taken at the beginning and end of foodservice operation on day

Table 1—Fatty acid oxidation and α-tocopherol loss during heat treatment of four different commercial frying oils (Experiment I)

Heat Treatments 177°C (350°F)	Frying oil α-tocopherol content[e] mg/100 g				Peroxide value of frying oil[e] meq/kg			
	I	II	III	IV	I	II	III	IV
1. Prior to heating	0.96	4.26[a]	4.17[a]	7.92[a]	0.56[ayz]	0.31[ax]	0.68[az]	0.46[axy]
2. Upon heating	0.87	3.76[b]	3.05[b]	6.80[b]	0.79[abxy]	0.73[bx]	0.69[az]	1.12[byz]
3. After heating 4 hr	0.72	3.22[c]	2.76[c]	6.49[c]	1.11[aby]	0.83[bcx]	1.35[abz]	1.24[bcyz]
4. After heating 8 hr	0.57	2.86[d]	2.49[d]	5.56[d]	1.23[bcy]	0.94[cx]	1.48[by]	1.35[bcy]
5. After heating 8 hr and cooling for 16 hr	0.52	2.68[d]	2.37[d]	4.64[e]	1.38[bcx]	1.31[dx]	1.59[by]	1.56[cdxy]
6. After heating 8 hr, cooling 16 hr and reheating 8 hr	0.26	2.52[f]	2.15[e]	3.70[f]	1.80[cy]	1.56[ex]	1.68[bxy]	1.73[dy]

[abcd] Within a column, means bearing different superscripts are significantly different ($P<0.05$).

[xyz] Within a row, means bearing different superscripts are significantly different ($P<0.05$).

[e] I = a partially hydrogenated soybean oil with methyl silicone added. II = a partially hydrogenated soybean oil with TBHQ, citric acid and dimethyl siloxane added. III = a semi-solid hydrogenated soybean and palm oil shortening with mono and diglycerides added. IV = 100% Corn Oil.

Table 2—Sampling of frying oil[a] and French fries, frying oil addition and product load during foodservice operation (Experiment II)

Sampling[b]	Time at 177°C (hours)	Frying oil addition[c] (liters)		Product load[d] Total wt (kg)		% as French fries	
		I	II[e]	I	II[e]	I	II[e]
1. Day 1 - Unheated oil and Uncooked French fries	0	8.8	8.8	58.5	77.0	75	71
2. Day 1 - Initial-First batch of French fries prepared	0	0	0				
3. Day 1 - Final-Last batch of French fries prepared	2	0	0				
4. Day 2 - Last batch of French fries prepared	4.5	0	1.3	58.5	83.3	75	74
5. Day 3 - Last batch of French fries prepared	7	0	0.9	40.6	75.5	82	65
6. Day 4 - Last batch of French fries prepared	9.5	3.0	0.7	44.4	75.5	75	68

[a] Frying Oil IV; a partially hydrogenated soybean oil with TBHQ, citric acid and dimethyl siloxane added.

[b] Hours of foodservice operation were from 11:00 am. to 1:30 p.m. daily.

[c] Oil was added "as needed" to replace oil taken up during product frying.

[d] French fries and onion rings were only products prepared in frying oil.

[e] I and II stand for two replications of the experiment performed 3 months apart.

1 and at the end of foodservice operation of the next 3 consecutive days.

Methods of analysis

Peroxide values, free fatty acids, vitamin C (reduced ascorbic acid) and proximate analysis of moisture and fat were determined in duplicate according to AOAC (1975).

Tocopherol was determined by reverse phase high pressure liquid chromatography (HPLC) on a Beckman 110A HPLC unit following Folch extraction of French fries (Folch and Lee, 1957), saponification and reconstitution of the samples in absolute ethanol. The saponification procedure used was as follows: An aliquot of 200 mg of extracted lipid was placed in a 20 mL screw cap tube. A volume of 1 mL 95% ethanol containing 0.02% each butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), 5.0% propyl gallate and 3.0 mL 5% KOH in ethanol were added. The tubes were flushed with N_2, capped and mixed on a vortex. Next the tubes were heated 10 min at 60–65°C and cooled under tap water. A volume of 1.5 mL H_2O and 3.0 mL hexane were added to each tube. The tubes were flushed with N_2, capped and inverted to mix the phases. The hexane phase was removed and the hexane extraction repeated with 2 mL hexane. The hexane extract was washed with 1.0 mL 5% glacial acetic acid. The hexane phase was removed to a glass tube and evaporated under N_2. The residue was redissolved in absolute ethanol for analysis by reverse phase HPLC.

Samples were analyzed on a Merck 12.5 cm C-18 reverse column. α-and α-tocopherol were detected by absorbance at 300 nm. D.L-α- and α-tocopherol standards (98% chromatographically pure, Sigma Chem. Co., St. Louis, MO) at a concentration of 20 μg/ml were used. The carrier was 98% methanol and 2% water with a flow rate of 1.2 mL per min. A Hewlett-Packard 3390A plotter integrater was used to record and integrate results.

Data analysis

Data were analyzed statist6ically by one-way randomized complete block ANOVA followed by multiple mean comparisons using Protected LSD. (Snedecor and Cochran, 1980).

RESULTS & DISCUSSION

Heat treatment of frying oils under laboratory conditions

Oxidation of frying oils. Free fatty acid (FFA) content and peroxide value (PV) of the oil were chosen as general indicators of deterioration of frying oil. Peroxides, despite their instability, are indicative of oil oxidation during frying through their formation in uniformly cooled samples (Fritsch et al., 1979; Fritsch, 1981). FFA were used as a measure of the effect of both oxidation and hydrolysis in FFA formation.

Significant differences in oxidation of the four frying oils were observed during different heating times. In all cases, fatty acid oxidation (peroxide values) during initial heat treatments (Treatment 2, Table 1) was significantly lower in fatty acid oxidation (peroxide values) than in the reheated oils (treatment 6, Table 1). Differences in the oxidation of the fats appeared to be associated with the following characteristics: presence of antioxidants, vitamin E content of the oil, degree of hydrogenation and unsaturation of the oil. The frying oil containing antioxidants (II) showed significantly less oxidation than the other oils after 4 and 8 hr heating (Treatments 3 and 4). The polyunsaturated corn oil (IV) underwent the greatest amount of fatty acid oxidation during the initial heating of the oil (Treatments 1 and 2). Despite its higher unsaturation and greater susceptibility to oxidation, the peroxide values of this oil were significantly different from the oil containing an antioxidant and a silicone derivative (II) only after heating. The oxidation of the corn oil was not as great as might be predicted. This protective effect may be due in part to its higher vitamin E content (Landers and Rathmann, 1981; Hildebrand et al., 1984). The rate of oxidation (the change between Treatments 5 and 6, Table 1) of the semi-solid hydrogenated vegetable oil (III) was lower during the final 8 hr of heating than the less hydro-

genated soybeans oils (I and II). The significant increase in oxidation of the partially hydrogenated soybean oil (I) which lacked factors to slow oxidation may be important if the oil is to be heated for a longer period of time.

Relationship of vitamin E loss to oxidation of frying oils

Vitamin E content of the frying oils was significantly different among all heat treatments (excluding Treatment 5) in 3 of the 4 frying oils studied (Table 1). Vitamin E loss of a heated fat occurred at different rates with oxidation (as indicated by peroxide values) in the various frying oils studied (Fig. 1). The relationship between oxidation of the fat and its vitamin E content appeared to be characteristic of each fat. The absolute value of the oxidation (peroxide value) cannot be related quantitatively to vitamin E loss in another fat. Oxidation effects on frying oil vitamin E appeared to be dependent upon other characteristics of the fat such as: initial vitamin E content, degree of hydrogenation and unsaturation of the fat and the presence of additives (Fig. 1). Antioxidant addition to a frying oil (II) may be most important in decreasing the rate of vitamin E loss at lower (PV < 0.73) and moderate (PV $>$ 0.94) oxidation levels (Fig. 1). Hydrogenation of soybean oil (III) appeared to most effectively control the rate of vitamin E loss during moderate oxidation (PV range 0.65–1.40). Both hydrogenation and addition of TBHQ have been shown to increase oxidative stability of oils (Erikson, 1983; Landers and Rathmann, 1981; Mounts et al., 1981). TBHQ addition has been shown to be more effective in enhancing frying oil stability than hydrogenation for deep fat frying applications (Buck, 1981). The partially hydrogenated soybean oil with no antioxidant (I) showed a constant rate of vitamin E loss with oxidation over a range of peroxide values (0.56–1.80). Overall, the corn oil (IV in Fig. 1) exhibited the greatest loss in vitamin E when peroxide values were greater than 1.00. Vitamin E (quantitative amounts) of the four fats studied are given in Table 1.

Vitamin E loss in heated frying oils

Vitamin E loss occurred nearly linearly over time in the four fats studied (Table 1). The loss during heating to 177°C (350°F) ranged from 9–27% (between Treatments 1 and 2, calculated from Table 1). Losses after heating at 177°C (350°F) for 8 hr (Treatment 4) ranged from 30–41% and cooling the oil in the fryer for 16 hr (Treatment 5) resulted in a further loss of 4–11%. Losses ranging from 41–73% occurred as a result of the entire heating process (Treatment 6). After the heat treatments differences in vitamin E of the frying oils were lessened (Table 1).

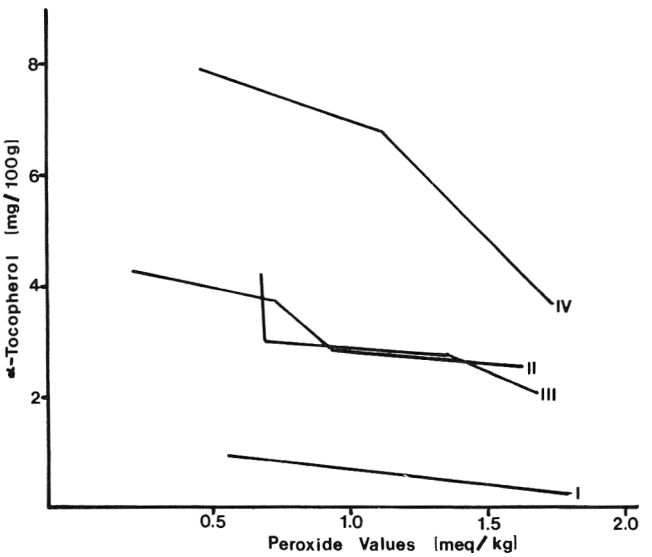

Fig. 1—Absolute α-tocopherol losses with oxidation. Numbers I to IV represent the frying oils; I = partially hydrogenated soybean oil with methyl silicone added; II = a partially hydrogenated soybean oil with TBHQ, citric acid and dimethyl siloxane added; III = a semi-solid hydrogenated soybean and palm oil shortening with mono and diglycerides added; IV = 100% corn oil.

Effects of experimental vs 'actual' operational use of frying oils on vitamin E content

Experimental conditions used to study nutrient losses occurring in foodservice operations must closely simulate actual foodservice practices. Surface effects, in particular, are extremely important in oxidative deterioration of oils (Kwon and Brown, 1984). The difference in data from model experiments versus "real" conditions demonstrate this. Bunnel et al. (1965) looked at the effect of heating oils on vitamin E loss under laboratory conditions. They heated 2.5 cm (1 in.) of frying oils in beakers for 3 hr at 204°C (400°F). Under these conditions, they found α-tocopherol losses of 96–99% in unhydrogenated and hydrogenated corn and soybean oils. In comparison by using "real" conditions (Table 1), losses of 24–34% of α-tocopherol occurred after heating 4 hr at 177°C (350°F). The greatest loss was 41% following 8 hr heating at this temperature. These studies were further pursued by examining vitamin E losses occurring in foodservice operations during the actual frying of foods in batch sizes common to the foodservice industry.

Frying oil used in actual foodservice operations

Oxidation and Vitamin E Losses of Frying Oil. During foodservice operation. α-tocopherol content of the frying oil (partially hydrogenated soybean oil with TBHQ, citric acid and dimethyl siloxane added) decreased with increases in heat treatment, fatty acid oxidation (PV) and free fatty acid formation (FFA) (Table 3). These losses were significantly different only for unheated oil (Treatment 1) and oil heated for more than 2 hr (Treatment 3, Tables 2 and 3). The largest loss of α-tocopherol occurred during the first day of use (Day 1 - Final, Table 3). There were no significant differences in γ-tocopherol among heating and holding treatments: however, additional oil was being added as is customary in the foodservice industry. This suggests that the practice of adding frying oil over the frying period can improve the vitamin E loss of the oil (Tables 2 and 3). α-Tocopherol was significantly different only for unheated and heated oils (Table 3). Changes in FFAs and PV of the oil over time were not significant when averaged for different replications (Table 3). Peroxide values increased more rapidly with greater product loads (replicates I and II, Tables 2 and 3). Increased oxidation of vegetable oils with prolonged deep fat frying is expected and has been indicated by other studies (Fritsch, 1981; Landers with Rathmann, 1981; Augustin and Berry, 1983).

Vitamin E in French fries with prolonged frying oil use

Although the α- and γ-tocopherols of the oil fell with increased usage (Table 3), there was no significant change in the α- or γ-tocopherol in the French fries over the period of frying oil use (Tables 3 and 4). The α-tocopherol of the French fries was similar to that reported by Bunnel et al. (1965). The effect of the decreasing α-tocopherol content of the oil on the french fry α-tocopherol content was improved by the significant increase in fat uptake by the fries over time (Table 4). The increased fat uptake by the food is associated with degeneration of the fat during foodservice operation despite proper use (Artman, 1969; Landers and Rathmann, 1981). These two factors: (1) decreasing vitamin E content of the oil, in spite of additions which contain vitamin E, and (2) increased fat uptake by the french fries with prolonged oil use, resulted in a lack

Table 3—Frying oil[d] deterioration and α, γ-tocopherol loss during foodservice operation (Experiment II)

Sample	Tocopherol (mg/100g) α	Tocopherol (mg/100g) γ	Free fatty acids[e,g] × 10^{-2}M	Peroxide value[e,g] (meq/kg)	Peroxide value[f,g] (meq/kg) I	Peroxide value[f,g] (meq/kg) II
1. Unheated	10.60[a]	57.24[a]	0.28	0.42	0.22 ± 0.03	0.61 ± 0.08
2. Day 1 - Initial	8.37[ab]	50.62[ab]	0.38	1.16	0.34 ± 0.16	1.97 ± 0.03
3. Day 1 - Final	7.26[b]	45.42[abc]	0.46	1.49	0.60 ± 0.03	2.38 ± 0.08
4. Day 2	6.79[b]	40.20[bc]	0.54	1.69	0.73 ± 0.03	2.64 ± 0.31
5. Day 3	6.27[b]	35.77[c]	1.25	2.91	1.11 ± 0.06	4.71 ± 0.08
6. Day 4	6.35[b]	35.39[c]	1.47	3.28	1.28 ± 0.01	5.08 ± 0.03

[abc] Within a column, means with the same superscript are not significantly different.
[d] A partially hydrogenated soybean oil with TBHQ, citric acid and dimethyl siloxane added.
[e] Mean of Replicates of the frying sequence; n = 2.
[f] I and II are replications of the frying sequence, Mean ± Std. dev., n = 3 for α,γ-tocopherol, n = 2 for peroxide values.
[g] No significant difference in means according to the F value determined by Completely Randomized Block ANOVA.

Table 4—% Moisture, fat and α,γ-tocopherol vitamin C of French fries prepared in "aging" frying oil[e] used in a foodservice operation (Experiment II)

Sample	% Moisture	% Fat	Vitamin C[f] mg/100g	Vitamin C[f] % AR[g]	Tocopherol[g] mg/100g[f] α	Tocopherol[g] mg/100g[f] γ	Tocopherol[g] % AR[h] α	Tocopherol[g] % AR[h] γ
1. Before Cooking	72.33[a]	5.34[a]	6.93[a]	100.00	0.06	0.36	100.00	100.00
2. Day 1 - Initial	53.79[b]	7.69[a]	6.73[a]	58.14	0.15	1.27	149.77	211.22
3. Day 1 - Final	50.01[bc]	10.95[b]	5.94[b]	47.43	0.14	0.89	129.03	136.82
4. Day 2	44.54[c]	12.42[bc]	5.23[bc]	37.64	0.14	1.31	116.13	181.55
5. Day 3	44.93[c]	14.35[cd]	4.80[c]	34.81	0.14	1.03	117.05	143.74
6. Day 4	44.17[c]	15.81[d]	3.97[d]	28.38	0.12	1.28	99.08	176.02

[a-d] Within a column, means with the same superscript are not significantly different according to analysis by Protected LSD test.
[e] A partially hydrogenated soybean oil with TBHQ, citric acid and dimethyl siloxane added.
[f] mg/100g wet weight of product.
[g] No significant difference in means according to the F Value determined by Completely Randomized Block ANOVA.
[h] % AR = % Apparent Retention = (Vitamin content/g cooked food (dry basis)/(Vitamin content/g raw food (dry basis).

of correlation between product tocopherol and deterioration of the frying oil.

Effect of prolonged frying oil use on vitamin C of French fries

The range of retention of ascorbic acid of 28–58% in French fries in similar to the ranges recorded in the literature (LaChance, 1975; Augustin et al., 1981). The low vitamin C content of the French fries may be a result of oxidation of the frozen product during storage and continued oxidation of the remaining ascorbic acid to dehydroascorbic acid (DHA) during deep fat frying. (Bunnel et al., 1965; Domah et al., 1974). The decrease in vitamin C and increase in fat may be of concern since they contribute to lower nutrient density of foodservice meals, a concern documented by Bunch (1984).

SUMMARY & CONCLUSIONS

THE RESULTS of this study suggested that the α-tocopherol content of frying oil can be influenced by: (1) type of food product (frying oil) selected for use; (2) duration of the use of the oil; and (3) addition of fresh oil during foodservice operations. However, when considering the loss of α-tocopherol in frying oil and the nutritional value of deep fat-fried foods, one must consider other factors. One such factor is increased fat uptake by the food product which occurs with increased use of the oil. In the present study, there were no significant differences in the vitamin E of French fries prepared in frying oil used over a 4- day time period. Although deterioration of the oil did not significantly affect the vitamin E of French fries prepared in the oil, it may have other nutritional consequences. Prolonged use of frying oil in a foodservice operation resulted in both a significant decrease in vitamin C and a significant increase of fat in the product, which may be very important to the consumer.

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Simultaneous Quantitative Determination of Manganese, Iron, Copper and Zinc by Atomic Absorption Spectroscopy in Tropical Cereals, Fruit and Legume Materials

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ABSTRACT

A method for the simultaneous quantitative determination of manganese, iron, copper and zinc in rice, corn, cassava, black beans, lettuce, mango, papaya, pineapple, medlar, bananas and melon by atomic absorption spectroscopy was developed by using a mixture of concentrated nitric acid-sulphuric acid (2:1 v/v) and by adding hydrogen peroxide for sample digestion. The precision varied between 1–10%. The accuracy was ascertained by analyzing five biological standard reference materials from the National Bureau of Standards (NBS, Washington, -DC. 20234 USA) and no significant difference could be established between the certified values and those present in this study. Additionally, the results obtained by the presented method were compared with those obtained by X-ray fluorescence spectrometry in a different laboratory.

INTRODUCTION

INTEREST in metallic elements in food has increased from the detection of gross criminal malpractices to investigations of chronic but possibly harmful effects on health of trace amounts present as contaminants. While the earth's crust remains the direct source of trace elements supplying the plant and animal media, today numerous external sources are modifying the natural cycle of trace elements in nature. Industrial, domestic and agricultural activities sometimes make use of large quantities of elements such as iron, copper, lead, nickel, cadmium or mercury, a part of which returns to the environment via discharges into the atmosphere, waters or by solid waste disposals (Crosby, 1977).

The importance of trace elements has also been recognized in the maintenance of optimum nutrition and health, as well as, in the beneficial effects of more complete information for use in nutritional labeling (Rockland et al., 1979). Such investigations require methods of analysis that are capable of measuring low levels of a wide variety of elements in different substrates (foods, feedingstuffs, animal and plant tissues).

Given the importance that certain trace elements play in the daily diet, the need for obtaining quick, precise and reliable values for those elements of nutritional interest has been emphasized. Several techniques are available for the determination of trace metals in different materials. Atomic absorption spectroscopy (AAS) has proved to be a valuable tool in agricultural, biological, and food analysis. In these fields it offers a rapid, convenient and reliable procedure for the estimations of toxic and nutritional metals in food and natural products. The main problems associated with trace metal estimation in organic materials by AAS are: (1) the quantitative extraction of the metallic species from the matrix; (2) the avoidance of mechanical losses; and (3) the avoidance of contamination from reagents (Thompson and Reynolds, 1978). Several digestion procedures have been used to dissolve these materials before analysis. Dry ashing involves the combustion of organic matter in a muffle furnace and subsequent dissolution of the constituents with acid. The possibility of losses and failure to dissolve the ash completely must be borne in mind. Acid digestion procedures incorporate oxidizing reagents to break down organic matter. The principal advantages of wet-oxidation methods are that they are applicable to a wide variety of samples, fairly rapid, less prone to either volatilization or retention losses and the liquid conditions are maintained. The usual wet digestion procedures for plant and food materials uses nitric-perchloric-sulphuric acid or nitric-perchloric acid mixtures (Arafat and Glooschenko, 1981). Oxidizing mixtures containing perchloric acid are in general unacceptable to some workers because of occasional reports of violent explosions. In addition to the hazardous nature of perchloric acid (Manufacturing Chemists Association, 1965), this acid can also produce losses of volatile elements, such as chromium (Gorsuch, 1962). Its use is undesirable and a procedure that obviated its use would improve the sample preparation. Another wet-ashing procedure in acidic (or alkaline) media is carried out under pressure in a PTFE-lined stainless steel vessel. (Crosby, 1977). The manufacturers claim that, by using this technique, the digestion is speeded up and volatilization losses are eliminated. There are limitations in using these devices, the primary disadvantage being the sample size (approximately 1g dry weight organic matter). If digestion of too large a sample is attempted, leakage and even explosions may occur owing to pressure build up in the decomposition vessel.

The mixture of sulphuric-nitric acid with the addition of hydrogen peroxide for sample digestion which was employed in the present investigation was already applied by Afrafat and Glooschenko (1981) for the simultaneous determination of arsenic, aluminum, iron, zinc, chromium and copper in mosses, lichen and higher plants.

The objective of this study was to develop an improved method for the quantitative determination of manganese, iron, copper, and zinc in tropical fruits.

MATERIALS & METHODS

Apparatus

A Varian Techtron AA-5 atomic absorption spectrometer coupled with a CRA-65 rod (Varian Techtron Pty Limited, Mulgrave, Victoria, Australia) was used. The absorption signals were recorded on a Yokogawa 3046 strip-chart recorder and on a Hewlett-Packard Model 5050-B printer (Hewlett-Packard Company, Palo Alto, CA). Single element hollow cathode lamps were employed. The instrument settings for each element were as recommended by the manufacturers and the gas mixture was adjusted in each case to give maximum signal response. For the analysis by X-ray fluorescence (XRF), a sequential X-ray spectrometer SRS 100 (Siemens A.G., D7500 Karlsruhe 21, West Germany) was employed.

Reagents

High purity certified reagents were used for all analyses: concentrated nitric acid, concentrated sulphuric acid and hydrogen peroxide, 30% v/v, (Merck, D-6100 Darmstadt, FRG). British Drug House (BDH) chemical stock solutions (1000 ppm) of iron, zinc, copper, and man-

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Table 1—Accuracy of the method

NBS standard reference material		Element			
		Fe (μg/g)	Mn (μg/g)	Zn (μg/g)	Cu (μg/g)
Wheat flour	Certified	18 ± 1	8.5 ± 0.5	11 ± 1	2.0 ± 0.3
(SRM 1567)	Found	20 ± 2	8.7 ± 0.2	11.6 ± 0.5	2.7 ± 0.1
Rice flour	Certified	8.7 ± 0.6	20.1 ± 0.4	19 ± 1	2.2 ± 0.3
(SRM 1568)	Found	8.8 ± 0.8	19.8 ± 0.6	21 ± 1	2.6 ± 0.3
Brewers yeast	Certified*	707	7	70	11
(SRM 1569)	Found	668 ± 37	6.7	70.4 ± 1.0	18.3 ± 0.4
Tomato leaves	Certified	690 ± 25	238 ± 7	62 ± 6	11 ± 1
(SRM 1573)	Found	705 ± 8	223 ± 5	69 ± 2	11.4 ± 0.7
Pine needles	Certified	200 ± 10	675 ± 15	60,72,52*	3.0 ± 0.3
(SRM 1575)	Found	219 ± 9	684 ± 18	79.9 ± 0.9	3.0 ± 0.3

* These values have been reported by the NBS but not certified

Table 2—Accuracy of X-ray fluorescence verified by addition of known concentration of standards

	Corn				Black Beans			
	Mn (μg/g)	Fe (μg/g)	Cu (μg/g)	Zn (μg/g)	Mn (μg/g)	Fe (μg/g)	Cu (μg/g)	Zn (μg/g)
Natural conc (NC) (by XRF)	7	24	2.5	21	16	102	10	29
Amount added	100	100	25	50	100	100	25	50
Detm conc (DC)	110 ± 11*	127 ± 11*	27.5 ± 5.4*	72.0 ± 4.8*	114 ± 11*	206 ± 11*	37.0 ± 5.4*	79.0 ± 4.8*
"Recovered" conc (RC = DC − NC)	103 ± 11*	103 ± 11*	25.0 ± 5.4*	51.0 ± 4.8*	98 ± 11*	102 ± 11*	27.0 ± 5.4*	50.0 ± 4.8*

* Distinction limits (Plesh, 1978) for a confidence level of 95% (Schorin and Piccioni 1984)

Table 3—Results of the nutritional elements in the investigated materials by atomic absorption spectroscopy (AAS) and X-ray fluorescence spectrometry (XRF)

Plant material		Mn (μg/g)	Fe (μg/g)	Cu (μg/g)	Zn (μg/g)
Rice	AAS	14.6 ± 0.7	117 ± 4	2.9 ± 0.2	17.88 ± 0.01
	XRF	13.7 ± 3.4	105.0 ± 3.4	3.1 ± 1.7	16.3 ± 1.5
Corn	AAS	12.9 ± 0.1	239 ± 14	1.85 ± 0.02	28.0 ± 0.4
	XRF	15.2 ± 3.4	216.0 ± 3.4	1.7 ± 1.7	25.0 ± 1.5
Cassava	AAS	35.5 ± 0.7	69 ± 3	2.6 ± 0.1	9.6 ± 0.1
	XRF	34.0 ± 3.4	71.0 ± 3.4	4.2 ± 1.7	8.6 ± 1.5
Lettuce	AAS	24.2 ± 0.3	913 ± 43	34.5 ± 0.7	78.8 ± 0.4
	XRF	n.d	n.d	n.d	n.d
Black Beans	AAS	21 ± 1	132 ± 2	10.7 ± 0.7	34.1 ± 0.6
	XRF	15.6 ± 3.4	114.0 ± 3.4	11.2 ± 1.7	33.0 ± 1.5
Mango	AAS	25.2 ± 0.4	77.6 ± 0.7	8.6 ± 0.4	3.2 ± 0.2
	XRF	25.0 ± 3.4	67.0 ± 3.4	9.0 ± 1.7	4.8 ± 1.5
Papaya	AAS	7.8 ± 0.2	58 ± 4	5.2 ± 0.2	17.8 ± 0.3
	XRF	10.1 ± 3.4	56.0 ± 3.4	7.2 ± 1.7	17.5 ± 1.5
Pineapple	AAS	135 ± 1	102 ± 2	8.9 ± 0.5	10.1 ± 1.0
	XRF	135.0 ± 3.4	84.0 ± 3.4	8.7 ± 1.7	9.7 ± 1.5
Medlar	AAS	2.7 ± 0.1	65 ± 3	2.2 ± 0.2	—
	XRF	3.5 ± 3.4	48.0 ± 3.4	2.7 ± 1.7	2.0 ± 1.5
Banana	AAS	3.43 ± 0.03	33 ± 2	5.4 ± 0.4	7.8 ± 0.3
	XRF	nd	nd	nd	nd
Melon	AAS	53 ± 3	111 ± 11	4.9 ± 0.5	28.4 ± 0.7
	XRF	21.0 ± 3.4	102.0 ± 3.4	3.7 ± 1.7	20.0 ± 1.5

nd = not determined

ganese were used to make the working standards. Deionized water (Milli-Q grade) was used to dilute samples and standards.

Sample preparation

The food samples obtained from commercial sources were cut to small pieces, if necessary, oven dried for several days at 80°C to constant weight, and then pulverized in a Spex Mixer Mill 800 (Spex Industries Inc, Metuchen, NJ) to less than 200 mesh (<74μm) using alumina ceramic cylinders. These containers were used to avoid contamination.

Sample digestion for atomic absorption determination

Two and a half grams of oven-dried, ground sample were placed into a 50 mL long neck beaker (made specially for this purpose), 5 mL of a mixture of concentrated nitric and sulphuric acid (2:1) was added with occasional stirring to obtain a homogeneous mixture and digested slowly at 70°C (approximately 1 hr) using a hot plate. Two more 5 mL portions of the acid mixture were added and the temperature was increased (100°C) and maintained until approximately 5 mL of solution remained.

The solution was allowed to cool (the cold digest was usually colorless or occasionally yellowish) and a few drops of hydrogen peroxide were added and heated gently. The heating was continued until the digest was clear. The final digested solution was transferred into a 50-mL calibrated flask and diluted to volume with deionized water. A blank digest was carried out in the same way. Most of the materials dissolved completely in the acid mixture, except for lettuce which produced a white precipitate (very little) during the digestion process. The precipitate was collected, dried at room temperature and further examined using an EDAX energy dispersive Si(Li) X-ray fluorescence analyzer (EDAX International, Prairie View, IL).

The results (Fig. 1) verified the presence of silica. Mineral quartz (silica) is known to be a constituent of some plant tissues and may therefore originate from the plant materials (Arafat and Glooschenko, 1981).

Analysis

Aliquots of the digest were analyzed for iron, zinc, and manganese by flame atomic absorption spectrometry using the standard addition method (Price, 1979). Copper was analyzed by electrothermal atomization (Fuller, 1977). To verify the accuracy of the applied analytical procedure, five NBS certified reference materials were digested to-

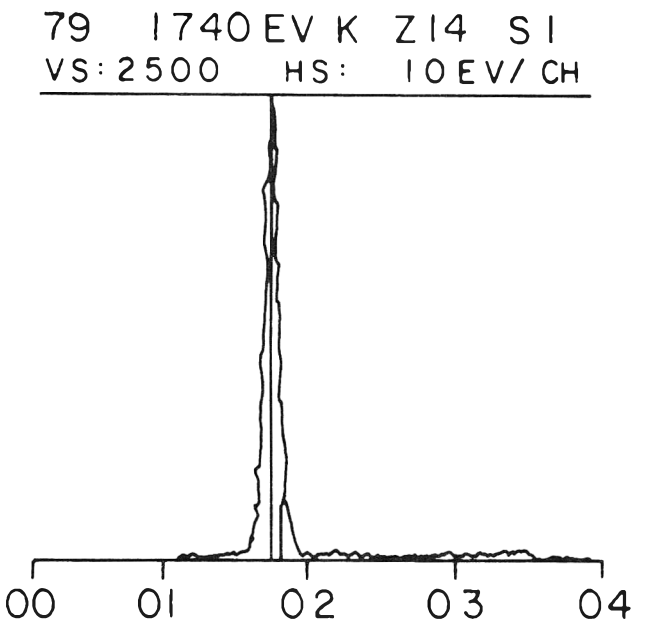

Fig. 1—Analysis of the residue from the sample digestion (lettuce) by energy dispersive X-ray fluorescence (EDX). [The meaning of the parameters shown on Fig. 1 are: the number 79 corresponds to the selected peak window number used; 1740 EV is the energy range of silicon; K is the principal emission line; Z14 indicates the atomic number of the element analyzed; SI is the element symbol; VS: 2500 describes the vertical scale used while HS: 10EV/CH indicates the hortizontal scale calibration (in the present case, it corresponds to 10 ev per channel).]

gether with the samples and analyzed quantitatively for Mn, Fe, Cu, and Zn under the same conditions: tomato leaves (SRM 1573), pine needles (SRM 1575), brewers yeast (SRM 1569), rice flour (SRM 1568), and wheat flour (SRM 1567).

Additionally, the accuracy of the method was evaluated by comparison of the analytical results obtained for the investigated cereals, fruits and legumes by atomic absorption spectroscopy with those attained by X-ray fluorescence spectrometry in a different laboratory.

The accuracy of the analysis by X-ray fluorescence spectrometry was verified on one hand by the analysis of the above mentioned five NBS biological standard reference materials (Schorin and Piccioni, 1984). On the other hand, it was possible to ascertain the accuracy by adding known concentrations of the elements to the materials investigated (Sachs, 1984). For this purpose 100 ppm of Mn and Fe, 50 ppm of Zn, and 25 ppm of Cu were added to four independent dried samples of corn as well as of black beans and analyzed.

RESULTS & DISCUSSION

THE ANALYTICAL RESULTS for the standard reference materials are contrasted with the certified values in Table 1. The standard deviations of the differences between the certified and obtained mean concentrations were then calculated (Sachs, 1984):

$$S_d^2 = S^2_{CV} - S^2_{AAS}$$

with S_d = standard deviation of the difference between two measuring series; S_{CV} = standard deviation given for the certified values; S_{AAS} = standard deviation of the mean concentrations obtained in this study.

Since all the deviations between the certified and analytical data ($\Delta = \overline{X}_{CV} - \overline{X}_{AAS}$) were smaller than the triple of the calculated standard deviation ($\Delta < 3s_d$), no distinction between the certified values and the results obtained by the present method could be established at the 99.7% confidence level. In Table 2, the results of the accuracy check for the XRF spectrometric method by the addition of known concentrations of the four elements to two investigated plant materials are presented. As can be inferred from this table, the deviations between the theoretical values and the "recovered" contents are smaller than the distinction limits (Plesch, 1978); therefore, the results have to be considered accurate.

The mean concentrations of the nutritional elements Fe, Cu, Zn, and Mn in the investigated fruits, cereals and legumes, which were obtained from three independent analysis by AAS, are summarized in Table 3. The standard deviations are also presented and give a measure of the precision of the method varying between 1–10%. For comparison purposes, the analytical results obtained by XRF spectrometry are also included in Table 3. Applying the same statistical analysis used to compare the standard reference materials, no significant differences between the results obtained by AAS and XRF were found for Cu and Zn. Only the results for Mn in melon were distinct, and different results were found for Fe in black beans, pineapple, and medlar.

CONCLUSIONS

THE NUTRITIONAL ELEMENTS Mn, Fe, Cu, and Zn — if present in the ppm range — can be determined precisely and accurately in tropical fruits, cereals and legumes by atomic absorption spectrometry employing a mixture of H_2SO_4-$HNO_3 + H_2O_2$ for sample digestion.

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A Research Note

Production of Citric Acid from Brewery Wastes by Surface Fermentation Using *Aspergillus niger*

T. ROUKAS and P. KOTZEKIDOU

ABSTRACT

The possible use of spent grain liquor and lager tank sediment as fermentation media for the production of citric acid by various *Aspergillus niger* strains has been investigated. A strain of *A. niger* ATCC 9142, on spent grain liquor, gave a citric acid concentration of 19 g/L, representing a yield of 78.5% (w/w) based on total reducing sugars consumed. On lager tank sediment 11.5 g/L citric acid were produced. The yield of citric acid, based on the total reducing sugars consumed, was 57.5% (w/w).

INTRODUCTION

THE FERMENTATIVE PROCESSES for the production of citric acid by surface and submerged methods using *Aspergillus niger* and different sources of carbohydrates (glucose, sucrose, beet and cane molasses, purified carob sugars, cotton waste and whey permeate) have been described (Prescott and Dunn, 1959; Marcris, 1975; Kiel et al., 1981; Hossain et al., 1983).

Recently, research has been directed to finding ways of utilizing brewery wastes. Spent grain liquor and lager tank sediment are significant sources of high-strength waste streams in the brewing industry. A number of brewery wastes were examined as substrates for fungal growth (Shannon and Stevenson, 1975). Hang et al. (1975) found *A. foetidus* (formerly called *A. niger* NRRL 337) capable of rapidly converting about 97% of the sugars from brewery spent grain liquor to fungal mass. This fungus produced a significant amount of citric acid during the submerged fermentation of brewery spent grain liquor (Hang et al., 1977).

The purpose of this investigation was to determine the ability of *A. niger* strains to produce citric acid in surface fermentation from both spent grain liquor and lager tank sediment.

MATERIALS & METHODS

Sources of wastes

Spent grain liquor (SGL) was the liquor resulting from the spent grain recovery process in a brewing plant. It contained the following, expressed as g/L: reducing sugars as glucose, 13.0; total reducing sugars as glucose, 25.7; pH 6.0.

Lager tank sediment (LTS) was the sludge obtained after wort fermentation and lagering of beer. It was centrifuged at 4,500 × *g* for 15 min and the supernatant used as fermentation medium. The liquor contained the following, expressed as g/L: reducing sugars as glucose, 4.0; total reducing sugars as glucose 31.5; pH 4.4

Organisms

The two strains of *Aspergillus niger* used throughout this investigation were *A. niger* ATCC 9142 and *A. niger* ATCC 10577. Both strains were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). The strains were maintained at 4°C on potato dextrose agar slants, and subcultured at intervals of from 1 to 4 months.

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Inoculum

Conidia suspensions for inoculations were obtained from cultures grown on potato dextrose agar slants at 30°C for 5 to 7 days. Spores were suspended in 5 mL sterile 0.8% Tween 80 solution and shaken vigorously for 1 min.

Fermentation medium

The experiments were carried out in 500 mL Erlenmeyer flasks containing 100 mL SGL or LTS. The flasks were autoclaved at 121°C for 15 min prior to use. After cooling, the pH was adjusted to 5.5 (Rokosu and Anenih, 1980; Chaudhary et al., 1978) with a sterile solution of 10% conc HCl or 1N NaOH. Each flask was inoculated with 0.5 mL of the inoculum and incubated at 30°C as surface fermentation.

Analytical techniques

At appropriate time intervals, fermentation flasks were removed and the contents analyzed.

Mycelial dry weight was determined by filtering, washing with distilled water and drying at 105°C to constant weight (ca 24 hr). Citric acid was determined by the method of Saffran and Denstedt (1948) using a Zeiss PMQII spectrophotometer. pH was measured using a Knick 646 pH meter equipped with a glass electrode. Total reducing sugars as glucose were determined by the method of Nelson (1944) after hydrolysis with conc HCl in a boiling water bath for 2 hr and neutralization with an equivalent amount of 10N NaOH (AOAC, 1975).

All samples were prepared in triplicate and the reported data are the average values.

RESULTS & DISCUSSION

A. NIGER STRAINS grown on spent grain liquor differed considerably in their capacity to produce citric acid. *A. niger* ATCC 9142 produced the highest concentration of citric acid, representing a yield of 78.5% on the basis of the total reducing sugars consumed, after 14 days of fermentation and then declined on the 18th day (Fig. 1a). The decline in productivity may have been due to a decay in the enzyme system responsible for the production of citric acid upon exhaustion of the fermentable sugars (Kristiansen and Sinclair, 1979). *A. niger* ATCC 10577 produced the highest concentration of citric acid, representing a yield of 49.4% on the basis of the total reducing sugars consumed, after 8 days of fermentation and then declined on the 14th day (Fig. 1a). Hang et al. (1977) reported that a high concentration of citric acid (12.3 g/L) was obtained when *A. foetidus* was grown on spent grain liquor in submerged fermentation after 96 hr incubation.

When *A. niger* strains were grown on lager tank sediment, citric acid accumulation started on about the 6th day and reached the maximum value on the 18th day (Fig. 1a). The yields of citric acid based on the total reducing sugars consumed, were 57.5% (ATCC 9142) and 47% (ATCC 10577). Szczodrak (1981) reported that a high concentration of citric acid (130 g/L) was obtained when *A. niger* I_{13} was grown on molasses medium in surface fermentation after 10 days incubation. Hossain et

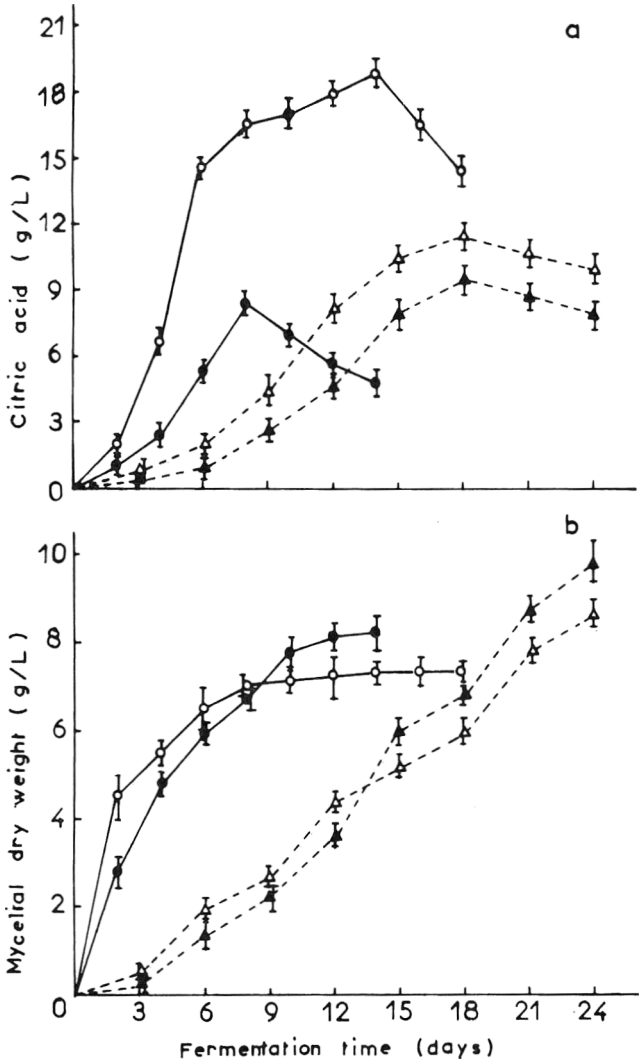

Fig. 1—Citric acid (a) and mycelial (b) production by A. niger surface cultures from spent grain liquor (SGL) and lager tank sediment (LTS). Each point is the mean ± SD of three separate experiments. ○——○ SGL *A. niger* ATCC 9142; ●——● SGL *A. niger* ATCC 10577; △- - - - -△ *LTS A. niger* ATCC 9142; ▲- - - - -▲ LTS *A. niger* ATCC 10577.

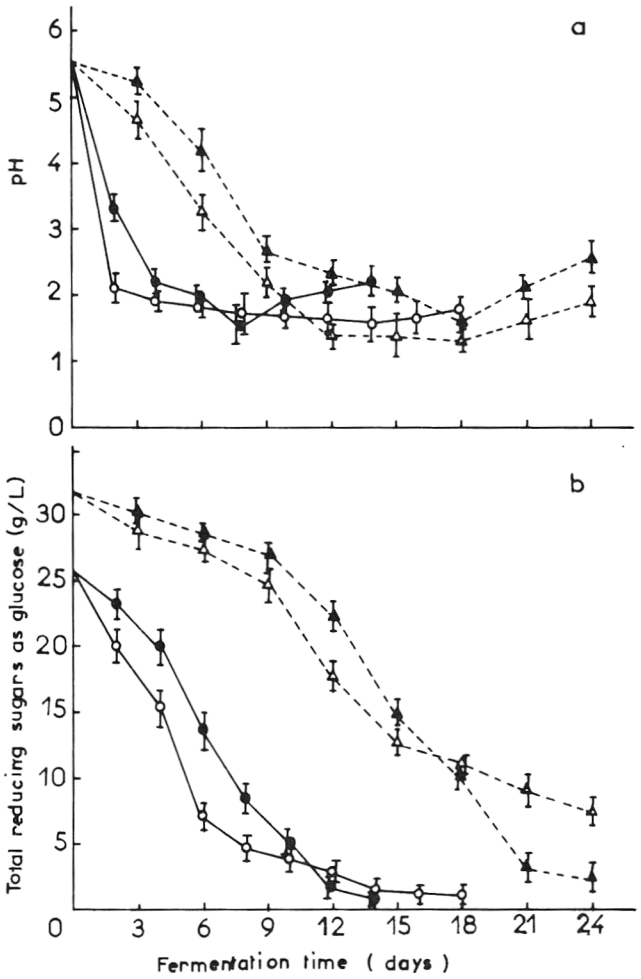

Fig. 2—pH values (a) and residual total reducing sugars (b) during citric acid fermentation by various A. niger strains in surface culture on spent grain liquor (SGL) and lager tank sediment (LTS). Each point is the mean ± SD of three separate experiments. ○——○ SGL *A. niger* ATCC 9142; ●——● SGL *A. niger* ATCC 10577; △- - - - -△ *LTS A. niger* ATCC 9142; ▲- - - - -▲ LTS *A. niger* ATCC 10577.

al. (1983) found that a high concentration of citric acid (8.3 g/L) was obtained when *A. niger* IMI 41874 was grown on whey permeate in submerged fermentation after 8 days incubation. There are several possible reasons for these differences including the strain of organism used, the nature of the sugar source, the other nutrients present and physical environmental factors such as dissolved oxygen tension.

Mycelial dry weight increased during fermentation (Fig. 1b). Both spent grain liquor and lager tank sediment were capable of supporting microbial growth, although there was a considerable difference in the yield obtained with each of the substrates. *A. niger* strains grown on spent grain liquor produced the greatest mycelial dry weight after 14 days of fermentation (Fig. 1b). When *A. niger* strains were grown on lager tank sediment the maximal yields were obtained after 24 days of fermentation (Fig. 1b). Shannon and Stevenson (1975) reported maximal yields of 6.28, 6.46, and 27.72 g/L for various fungal strains grown on grain press liquor, fermentation sludge liquor and trub press liquor, respectively. Hang et al. (1977) reported a maximal yield of 11 g/L for *A. foetidus* grown on spent grain liquor in submerged fermentation after 96 hr incubation.

The pH decreased during fermentation (Fig. 2a). This was due to the citric acid production during fermentation of reducing sugars. The lowest value of pH was accompanied with the greatest concentration of citric acid (Fig. 1a and 2a). Then the pH value increased due to oxidation of citric acid by the fungus (Hang et al., 1975).

The total reducing sugars decreased during fermentation (Fig. 2b). When the amount of citric acid increased in the medium, the level of sugars fell to trace levels.

Results show that *A. niger* ATCC 9142 produced more citric acid than *A. niger* ATCC 10577 and that both spent grain liquor and lager tank sediment can be used as fermentation media.

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A Research Note

Determination and Correlation of the Water Activity of Cheese Whey Solutions

ROSA J. KANTEREWICZ and JORGE CHIRIFE

ABSTRACT

This work reports on the experimental determination (and correlation) of water activity (a_w) in cheese whey solutions of varying total solids content (up to about 50% solids). It was shown that a satisfactory linear correlation between a_w and solids (given as g of solids/100g of water) of whey existed. The a_w of whey solutions was close to but always below that of lactose, which indicated that other nonlactose constituents also contributed to a_w lowering.

INTRODUCTION

RECENTLY, Kanterewicz et al. (1985) developed a simple process to achieve microbial stability of cheese whey stored at ambient temperature. This process was based on a combination of reduced water activity (a_w), pH, and addition of potassium sorbate; the stabilized whey was stored up to 3 months at 30°C without bacterial or mold deterioration.

Little is known about the water activity of whey solutions. This work reports on the experimental determination and mathematical correlation of a_w in solutions of whey of varied concentrations.

MATERIALS & METHODS

Materials

Concentrated sweet whey, supplied by SANCOR Cooperativas Unidas Ltd. (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. Whey solutions of varying solids content were prepared by dilution of the concentrated product with distilled water.

Determination of water activity

The a_w of whey samples with a value above 0.975 was derived from measurement of the freezing point. Freezing points were determined on an Advanced Instruments Milk Cryoscope 4D II (Needham Heights, MA) which was calibrated against standard NaCl solutions. All measurements were made in duplicate and the average was used.

The water activity of the samples with a value below 0.975 was determined using the "a_w-Wert Messer" manufactured by firma LUFFT Stuttgart (West Germany). In order to improve the reliability of the measurements, the instrument was operated following the procedure of Chirife and Ferro Fontán (1980). 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 measurements were made at 25 ± 0.2°C.

RESULTS & DISCUSSION

THE LOWERING of freezing point of whey is a function of the concentration and chemical nature of the dissolved solids and is thermodynamically related to a_w. The initial freezing temperature of whey as a function of solid content was measured and a_w was calculated according to Eq. (1) (Ferro Fontán and Chirife, 1981)

$$-\ln a_w = 9.6934\ 10^{-3}\ \theta_F + 4.761\ 10^{-6}\ \theta_F^2 \qquad (1)$$

where θ_F is the freezing point depression. Recent work by various workers (Miracco et al., 1981; Chirife et al., 1981) demonstrated that cryoscopic measurements constitute an accurate and convenient way to measure the a_w of high a_w solutions.

The a_w of whey solutions are compared with values calculated from freezing point data reported by Bakshi and Johnson (1983) for reconstituted spray-dried sweet whey, and by Radewonuk et al. (1983) for reconstituted nonfat dry milk (Fig. 1). Whey samples had 73–75% lactose on a dry solid basis, while the nonfat milk sample had 51.8% lactose on the same basis. All the data had approximately the same correlation when a_w was plotted as a function of the ratio lactose/water in different whey and milk solutions. Lactose plays a major role in a_w lowering in whey or milk; however, other soluble solids also help to depress a_w. This is best illustrated in Fig. 2 which compares the a_w lowering behavior of cheese whey up to about 50% (w/w) solids concentration, with that predicted for lactose solutions having the same ratio for lactose/water in corresponding whey solutions. The a_w of lactose solutions was obtained from the following equation (Miracco et al., 1981):

$$a_w = X_1 \exp(-10.2\ X_2^2) \qquad (2)$$

where X_1 and X_2 are molar fractions of water and lactose. The predicted curve for lactose was computed on the basis that the whey contained 73% lactose on a dry solid basis. The a_w of whey solutions was close to but always below that of lactose, which indicated that other nonlactose constituents also contributed to a_w lowering. Dry sweet whey contains about 8% ash whose major components are calcium, phosphorus, magnesium, sodium, and potassium (Bakshi and Johnson, 1983). Since these elements have relatively low atomic weights, as compared to lactose, they also contribute to a_w lowering. Also shown in Fig. 2, a satisfactory linear correlation between a_w and solids (given as g of solids/100g of water) of whey exists. The line corresponds to the equation (correlation coefficient is 0.9940).

$$(a_w)_{whey} = 0.999 - 0.000558\ M \qquad (3)$$

where M is the solids content in g solids/100g of water. This linear relationship can be based on a known simplified thermodynamic relation for the a_w of a binary solution (Lupin et al., 1981),

$$a_w \cong 1 - (\phi \cdot 0.018\nu)m \qquad (4)$$

where ϕ is the osmotic coefficient; ν is the number of moles of all species which give 1 mole of solute in solution ($\nu = 1$ for nonelectrolytes) and m is the molality. The osmotic coefficient varies with molality; however, in certain ranges the changes it undergoes may be small and Eq. (4) can be written:

$$a_w \cong 1 - K\ m$$

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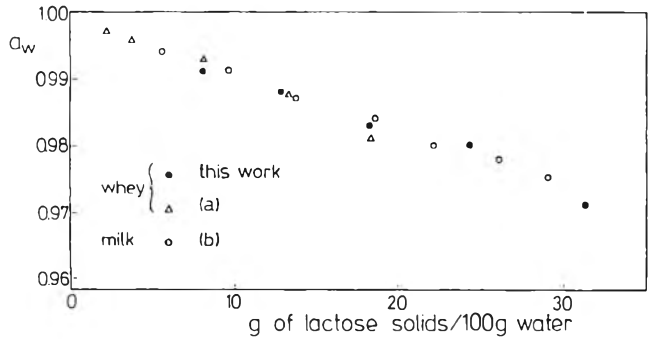

Fig. 1—Comparison of water activity (derived from freezing point measurements) of sweet whey and nonfat milk solution; (a) Bakshi and Johnson (1983); (b) Radewonuk et al. (1983).

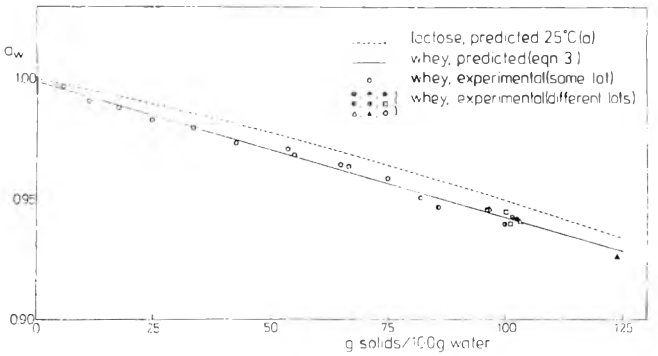

Fig. 2—The water activity of sweet whey solutions. Values of $a_w \geq 0.974$ were derived from measured freezing points; values < 0.975 were measured with the hygrometer.

(5)

where $K = (\phi \cdot 0.018\nu)$. Eq. (5) is almost identical to Eq. (3).

As shown here, lactose plays a major role in a_w lowering in concentrated whey solutions having up to about 125g solids/100g water. Lactose concentration in the aqueous phase of whey of this concentration amounts to about 91g lactose/100g water. The solubility of lactose at 25°C has been reported to be only 20.5–21.7g lactose/100g water (Nickerson and Moore, 1972; Jelen and Coulter, 1973). Thus, lactose might exist in a supersaturated condition in our concentrated whey solutions. However, lactose crystallization was not observed in concentrated whey samples ($a_w \approx 0.94$) stored for up to 3 months at 30°C. The a_w also remained unchanged during storage confirming that lactose crystallization did not occur. The stability of this supersaturated lactose condition may be attributed to the presence of proteins in the whey which cause an excessive viscosity that interferes with the crystallization of lactose (Nickerson, 1977). It was calculated that the viscosity of a whey solution having $a_w \cong 0.94$ (about 100g solids/100g water) was several times larger than that of a lactose solution having an equivalent lactose concentration (Buma, 1980). Pure concentrated lactose solutions were prepared in the laboratory by dissolving lactose crystals in water held at elevated temperatures. In contrast to concentrated whey solutions, lactose crystals spontaneously formed during cooling and standing at room temperature. Thus, the high viscosity may be a reason, among others, for the lack of lactose crystallization in concentrated whey solutions.

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A Research Note

Acetaldehyde and Accelerated Storage of Wine: A New Rapid Method for Analysis

J.S. JONES, G.D. SADLER, and P.E. NELSON

ABSTRACT

A method was developed for rapid analysis of total acetaldehyde in wine. The method converts acetaldehyde acetals and bisulfite addition products to free acetaldehyde. These conversions were achieved through a series of 1 min acid, base, and iodine treatments followed by a 10 min equilibration period. Acetaldehyde was quantified by gas chromatographic headspace analysis and the method was found to be precise to ± 1 ppm standard deviation. An accelerated shelf life procedure using this method was developed by promoting oxidation by agitation at oxygen saturation. The resulting procedure was 4 to 15 times faster than previous studies.

INTRODUCTION

FOR CENTURIES the wine industry has tried to minimize oxygen contact with wines because of undesirable changes in color, flavor, and bouquet. However, with the recent packaging revolution, wineries have begun bottling in containers made of oxygen-permeable membranes such as polyester-polyethylene-foil-paper laminants. This exposure to oxygen, whether it occurs through plastic bottles, bag and box, laminants, or through plastic pipes and fittings, has created a need for rapid methods of analysis and prediction of oxidative degradation. Current accelerated shelf life study methods are unsatisfactory. The elevated temperature shelf life studies are unrepresentative of the true shelf life because of the high volatility (b.p. 20.8°C) of acetaldehyde and the direct proportional dependence of acetaldehyde production on temperature (Tolmachev et al., 1982). Other studies (Joslyn and Comar, 1941) which take nearly a year to complete are too long to be of substantive benefit to industry.

Autoxidation of wines is a complex process which is not completely understood. It involves catalytic heavy metals (Wildenradt and Singleton, 1974) in a reaction cascade that is thought to begin with the oxidation of phenolic compounds, primarily anthocyanins, catechines, and tannins (Singleton and Noble, 1976). The cascade eventually leads to the oxidation of ethanol to acetaldehyde by a strong oxidant thought to be hydrogen peroxide (Wildenradt and Singleton, 1974). As the wine ages, anthocyanins nearly disappear by copolymerizing with phenolic compounds in the presence of acetaldehyde (Ribereau-Gayon et al., 1983). The highly colored state of the condensed anthocyanin results in the darkening of the wines. As phenolic polymerization progresses from monomers to larger oligomers bitterness decreases and astringency increases (Amerine et al., 1980). When the polymer size becomes too large to be held in solution, precipitation with concurrent decrease in color and anthocyanin occurs (Ribereau-Gayon et al., 1983).

The analysis of acetaldehyde concentration in wine is confounded by acetaldehyde complexing reactions. The primary mode of acetaldehyde disappearance is through bisulfite complexation, but it also results from reaction with phenols and binding with alcohols and aldehydes to form acetals (Joslyn and Comar, 1941).

Of the many methods available for acetaldehyde determination, only the AOAC titration method (1980) measures acetaldehyde as acetals, bisulfite addition products, and free acetaldehyde. This method is very time consuming, requiring several 15-min reaction periods as well as distillation and titration steps. The purpose of this investigation was to develop a rapid analysis for total acetaldehyde not otherwise reacted.

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MATERIALS & METHODS

Total acetaldehyde

The acid, base, and iodine treatments were based on the same principles as the AOAC (1980) method for acetaldehyde in wine. Acetals were hydrolyzed to their aldehydes and alcohols by acidification for 1 min of a 5 mL 25°C wine sample with the addition of 1 mL 20% HCl. Cleavage of the acetaldehyde-bisulfite addition product was achieved by an alkaline treatment for 1 min with the addition of 1 mL Na_3BO_3 solution (10g H_3BO_3 + 17g NaOH diluted with H_2O to 100 mL). The pH of the sodium borate solution was adjusted with HCl to provide for an alkaline treatment at pH ~ 9. Recomplexation of bisulfite with acetaldehyde was prevented by addition of 10 drops 0.1N KI solution immediately following the Na_3BO_3 addition. The bisulfite elimination end point was verified by the characteristic blue color of a 0.2% starch indicator. The sample was neutralized prior to the equilibration step to prevent further alkaline reactions. Neutralization of the alkaline sample was achieved by addition of 9–10 drops 20% HCl. The treated wine was allowed to equilibrate for 10 min in a septum-sealed 25 mL Erlenmeyer flask while submerged in a 25°C controlled temperature water bath.

The headspace gas chromatographic procedure was based on a method used by Mendenhall et al. (1980). A Perkin-Elmer Sigma 1 gas chromatograph was used with N_2 as the carrier gas at a flow rate of 30 mL/min. Using a pre-warmed 5 mL gas tight syringe, a 3 mL headspace sample was injected in to the injection port which was maintained at 100°C. A glass (6 ft, 2 mm i.d., 1/4 in o.d.) Porapak Q 100–120 mesh column was used with an oven setting of 130°C and a flame ionization detector setting of 195°C. Injections taken from the headspace above 0.01% standard acetaldehyde solutions provided the response factor for calibration.

Accelerated shelf life study

Six 950 mL, brown glass, Saran-sealed, screw-cap bottles containing 200 mL wine and 0.05% sodium benzoate as microbial inhibitor were constantly agitated in a covered 25°C controlled temperature water bath. The wine was maintained at oxygen saturation (6–7 mL/L) with oxygen derived from the air in the headspace (Singleton et al., 1979). Once every 2–7 days a shelf life study bottle was removed, cooled to 10°C, and swirled to recover condensate. A portion of the chilled wine was then dispensed into a zero headspace vial. Immediately prior to removal of 5 mL for acetaldehyde analysis, the temperature of the vial of wine was raised to 25°C in a water bath.

Three shelf life studies were performed — one with white wine and two with red wines. All three wines were bottled within 6 months of the time of this investigation.

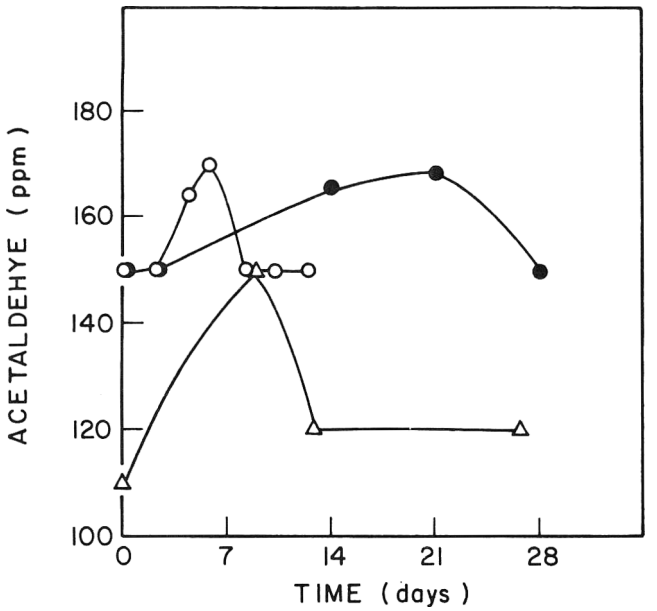

Fig. 1—Effect on acetaldehyde concentration of a shelf life study using agitation at oxygen saturation to accelerate oxidative degradation reactions in (●) a white wine and (○) (△) two red wines.

RESULTS & DISCUSSION

Total acetaldehyde

Gas chromatographic quantification generated a baseline-resolved acetaldehyde peak with a retention time of approximately 2.5 minutes. Ethanol and other extraneous compounds eluted quickly, enabling the injection of one sample every 10 minutes. The precision of the acetaldehyde analysis was excellent. Twenty duplicate tests, where the range was between 100 and 180 ppm acetaldehyde, gave a mean difference of 2.3 ± 1 ppm standard deviation.

This method of total acetaldehyde analysis was more than three times faster than the AOAC titration method.

Accelerated shelf life study

The acetaldehyde concentration in white wine reached a maximum value after 21 days; thereafter it declined sharply (Fig. 1). Studies with red wine showed similar concentration patterns; however, the rate of acetaldehyde formation was greatest after only 7–9 days.

The accelerated shelf life study described provides a rapid means by which the acetaldehyde production and consumption pattern of different wines can be predicted. This study can be completed within a week to a month compared to a year in previous studies.

Current shelf life and taste panel studies involve too much time and money. Wineries need to know the oxidation susceptibility of each type of wine in order to decide the package barrier qualities necessary to attain a certain shelf life. If wineries could predict quality with respect to oxidation in advance, the wines could be marketed at the time of highest quality. Wineries have also expressed interest in using low cost plastics as a replacement for stainless steel pipes and bungs.

The method outlined herein provides a rapid objective means by which acetaldehyde concentration can be monitored. Future research using this method in conjunction with new oxygen consumption devices may lead to a kinetic relationship between oxygen consumption and acetaldehyde production whereby the acetaldehyde concentrations could be used to calculate the permeability of membranes in contact with wine.

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A Research Note

Effect of Treatment, Ice Storage and Freezing on Residual Sulfite in Shrimp

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ABSTRACT

When fresh shell-on tails of penaeid shrimp were treated with sodium bisulfite according to current good manufacturing practices, residual sulfite (SO_2) on the edible portion varied from a high of 92.7 to a low of 60.9 ppm with an average of 80.2 ppm. During freezing and a short period of frozen storage, there was an average loss of 17% in residual sulfite. For shrimp held on ice storage, on the other hand, there was a rapid decrease in residual sulfite to below 10 ppm after six days of storage. The residual levels found on treated shrimp were consistent with action levels set by USFDA for shrimp treated according to good manufacturing practices.

INTRODUCTION

TO INHIBIT black discolorations on the shell of shrimp, freshly harvested raw shrimp have for years been dipped in diluted solutions of sodium bisulfite immediately after harvest. The United States Food and Drug Administration (USFDA) has recognized the need for this additive in the shrimp industry and regards a 1 min dip in a 1.25% solution of sodium bisulfite as current good manufacturing practice (CGMP).

Recently the safety of sulfiting agents has been questioned in the clinical literature and by consumer groups. A number of reports have described strong asthmatic reactions to bisulfite and other sulfiting agents (Allen and Collett, 1981; Stevenson and Simon, 1981a, b; Baker et al., 1981; Baker and Allen, 1982; Werth, 1982; Simon et al., 1982). Due to the potential adverse health problems associated with sulfiting agents, the USFDA initiated a recall for imported Spanish Red Shrimp containing more than 300 parts per million (ppm) sulfur dioxide. Such high levels indicate a lack of good manufacturing practices (Anon., 1984).

Since only a limited amount of information is available regarding residual levels of sulfite in shrimp, the purpose of this study was to determine what levels would result by treating shrimp according to the CGMP and how these levels are affected by freezing and by ice storage.

MATERIALS & METHODS

THREE SPECIES of penaeid shrimp [brown (*Penaeus aztecus*), white (*P. setiferus*), and pink (*P. duorarum*)] of different sizes harvested on the Texas Coast in the Gulf of Mexico were deheaded and dipped in a 1.25% solution of sodium bisulfite at a ratio of 454g shrimp to 2L for exactly 1 min and rinsed for 15 sec using a spray hose. This treatment is part of the CGMP's for shrimp processing. Immediately after treatment, duplicate samples weighing approximately 500g from each treated lot were peeled and analyzed for residual sulfite while the remainder was frozen in a blast freezer at −26°C as 5-lb portions in appropriate wax-impregnated cardboard boxes. After 24 hr, the packed boxes were removed from the freezer and water was added to form a uniform solid glaze. The 5-lb shrimp-blocks were placed back in the freezer and kept at −26°C for approximately 1 wk. Upon removal from frozen storage, the blocks were left to thaw in a cold-room at 4°C. Two 500g core samples of shrimp were removed from each block, peeled and 100g analyzed for residual sulfite.

Three 10-lb lots of brown shrimp treated with sodium bisulfite as described above were mixed with ice at a ratio of 1:2 and placed in well insulated ice chests. Immediately after treatment and at regular time intervals during the ice storage period, the shrimp were analyzed in triplicate for residual sulfite both on the shell-on and peeled portions. The dip volume from the melting ice was also collected, measured, and analyzed for sulfite.

Residual sulfite was determined on 100g samples according to the method of Monier-Williams (AOAC, No. 20.106, 1980). The accuracy of the analytical procedure was monitored regularly by determining the sulfite content of analytical grade sodium bisulfite.

The data were statistically analyzed using analysis of variance (ANOVA) at the Data Processing Center of Texas A&M University. Data that showed significant differences were further analyzed using Duncan's multiple range test.

RESULTS & DISCUSSION

TABLE 1 shows residual levels of sulfite in shrimp treated according to current good manufacturing practice and kept in frozen storage for 7 to 10 days. The initial SO_2 concentrations were higher than those reported by other investigators for domestically produced shrimp. Wood et al. (1976) analyzed 80 commercially available samples from seven countries, including 40 domestically produced, and found extreme variation from nondetectable to as high as 1752 ppm sulfite on the shell of one sample imported from Trinidad. However, apart from one sample which contained 112 ppm on the edible part, sulfite residues in domestically produced shrimp were low or could not be detected. Weingartner et al. (1977) dipped fresh headless brown shrimp in both 1.25% and 5% solutions of sodium bisulfite and placed them on ice for 15 days. In spite of the

Table 1—Residual sulfite (ppm SO_2) in shrimp after treatment and freezing

Species	Count size (tails/lb)	Treatment weight	Residual conc ppm SO_2 Initial[a]	After freezing	N[b]
Pink	36 — 40	6 lb	92.1 ± 1.1	64.4 ± 3.2	2
Pink	60 — 70	12 lb	70.2 ± 10.8	68.5 ± 4.7	4
Pink	70 — 100	6 lb	75.6 ± 10.6	56.1 ± 0.0	2
White	21 — 25	6 lb	83.9 ± 1.3	68.1 ± 3.4	4
White	21 — 25	12 lb	88.4 ± 11.7	73.4 ± 8.8	4
White	50 — 60	6 lb	60.9 ± 2.3	69.8 ± 0.9	2
Brown	10 — 15	18 lb	92.7 ± 11.1	66.5 ± 11.6	6
Brown	60 — 70	12 lb	85.7 ± 14.7	76.2 ± 4.5	4
Brown	70 — 100	6 lb	72.5 ± 3.9	54.0 ± 3.0	2
		Average	80.2 ± 11.0	66.3 ± 7.3	

[a] Average of duplicate samples

[b] N = Number of replicates on frozen samples.

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Table 2—Residual sulfite (ppm SO_2) in treated[e] brown shrimp during storage on ice

Days on ice	Size 61–70 tails/lb		Size 51–60 tails/lb		Size 31–35 tails/lb	
	Edible portion	Shell-on	Edible portion	Shell-on	Edible portion	Shell-on
0	82.0 ± 8.0[a]	125.4 ± 13.6[a]	77.6 ± 2.1[a]	211.4 ± 37.8[a]	62.1 ± 4.3[a]	92.2 ± 11.9[a]
1		66.2 ± 13.1[b]		98.3 ± 11.4[b]		39.2 ± 3.8[b]
2	27.4 ± 3.1[b]		35.9 ± 6.0[b]		17.4 ± 2.1[b]	
3		29.1 ± 2.3[b]		49.6 ± 1.8[c]		20.0 ± 2.9[c]
4	4.6 ± 1.0[c]		18.4 ± 3.3[c]		4.3 ± 0.8[c]	
5		15.7 ± 2.0[cd]		22.6 ± 4.0[c]		14.3 ± 2.2[c]
6	3.0 ± 1.6[c]		4.3 ± 0.5[d]		2.0 ± 1.1[c]	
7		9.9 ± 2.0[d]		17.8 ± 2.2[d]		

[a–d] Values in the same column with differing superscript are significantly different at $p<0.05$.

[e] Shell-on shrimp-tails were dipped in 1.25% solution of sodium bisulfite (454g shrimp/2L solution) for 1 min and placed in ice at a ratio of one part shrimp to two parts ice (w/w).

long dip-time, they found low residual levels with a high of only 25 ppm sulfite.

When shrimp were frozen in 5-lb blocks, the average SO_2 concentration dropped approximately 17% (Table 1). Since the loss of sulfite was independent of the time in frozen storage, the washing effect of the thaw drip was the most likely cause for the reduction in residual sulfite.

Table 2 shows residual sulfite in both the edible and shell-on portions of three separate lots of shrimp-tails treated according to CGMP and held on ice for 1 wk. Immediately after treatment, the range for residual sulfite levels was in agreement with the data presented in Table 1. The majority of the additive picked up by the shrimp appears to be associated with the shell. Even though the shell-weight is less than 5% of the total tail-weight, unpeeled shrimp contained much higher levels than did peeled shrimp. After 2 days of ice storage, the concentration of residual sulfite in all three lots was less than half the original concentration on the shrimp. Since the reduction of sulfite in the shrimp could be stoichiometrically accounted for in the drip, the rapid loss of sulfite was due to the leaching effect of the melting ice. On the sixth day of storage, all samples had less than 10 ppm on the edible portion, which from a labeling standpoint, is important (Anon. 1985).

For shrimp treated with sodium bisulfite according to prescribed procedures and placed on ice, low levels of residual sulfite are to be expected at the time the shrimp are unloaded. However, for shrimp treated and frozen, residual sulfite on the edible portion will fall between 50 and 100 ppm. These levels are consistent with action levels set by the USFDA which regard shrimp products containing over 100 ppm on the edible portion as adulterated and potentially hazardous to health.

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A Research Note

Effects of Experimental Cooking on the Yield and Proximate Composition of Three Selected Legumes

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ABSTRACT

Dry mature seeds of field pea (*Pisum arvense*), moth bean (*Vigna aconitifolia*) and pigeon pea (*Cajanus cajan*) were investigated for their chemical and cooking characteristics. The crude protein (N x 6.25) content was 24.5% for field pea (8.5% moisture basis), 22.7% for moth beans (10% moisture basis) and 21.3% for pigeon pea (10% moisture basis). Ratios of cooked weight/dry weight after cooking the whole and dehulled seeds were 2.14 and 2.18, 2.20 and 2.30, and 2.43 and 2.51 for the field pea, moth bean and pigeon pea, respectively. Dehulling increased the protein, fat and energy contents but decreased the ash and fiber contents as well as cooking times. On cooking, the true retentions of proximate principles were good.

INTRODUCTION

DRY MATURE LEGUME SEEDS form an important component of the diets in many developing countries and are consumed in large quantities in the Middle Eastern Countries (Kuzayli et al., 1966; Akroyd and Doughty, 1969). In the Kingdom of Saudi Arabia, several legumes are imported for human consumption. To increase the local production, a number of legumes are currently studied for cultivation under the local environmental conditions. Field pea (*Pisum arvense* L.), moth beans (*Vigna aconitifolia* [Jacq.] Marechal, Syn; *Phaseolus aconitifolius*) and pigeon pea (*Cajanus cajan* (L) Millsp.) hold great potential for thriving under the local environmental conditions because of their successful cultivation in other countries with environmental conditions similar to those of Saudi Arabia (Tikka et al., 1973, ICRISAT, 1981). The use of these legume seeds in human diets will largely depend on their nutritional quality and cooking behavior. In the literature, there is limited information on these aspects about the paired samples (raw and cooked) of these legumes. The present investigation was, therefore, undertaken to study the effects of experimental cooking on the yield and proximate composition of whole and dehulled seeds of field pea, moth bean and pigeon pea.

MATERIALS & METHODS

TWO LOTS of dry mature seeds of field pea, moth bean and pigeon pea were purchased from a market in Peshawar, Pakistan. After washing and drying at room temperature, half of each lot was dehulled manually. Half of whole seeds and the dehulled seeds (split cotyledons, also called 'dhal') were kept for cooking experiments while the remaining were ground in a laboratory micro-mill to pass a 1 mm sieve. For cookability test, approximately 5g of each legume were cooked in 50 mL deionized water. The cooking time was determined when the grains or dhals were tender, as assessed by pressing the cooked samples between two glass slides so that no hard material was found (Narasimha and Desikachar, 1978). When cooked, the legumes were strained and their weights and volumes recorded. After determining their moisture content, the cooked legumes were dried and ground as before. The cooking water from each sample was adjusted to 50 mL and retained for analysis. Percent solids dispersed in cooking water were determined as: 100 X dry matter in 50 mL cooking water, divided by weight of the sample.

Proximate analysis of the legume samples and cooking water was done according to AOAC (1980). Crude protein (N x 6.25) was determined with an automatic analyzer (Kjel-Foss Automatic 16210, A/SN. Foss Electric, Denmark) (AOAC, 1980). Energy contents were calculated using factors of 4, 9 and 4 kcals/g for protein, fat and NFE respectively, and also expressed in megajoules (MJ) by multiplying the kcals by the factor 0.004184.

The following formula of Murphy et al. (1975) was used to calculate the true retention (TR) of a nutrient:

$$TR(\%) = \frac{\text{nutrient content/g of cooked food} \times \text{g of food after cooking}}{\text{nutrient content/g of raw food} \times \text{g of food before cooking}} \times 100$$

Data were analyzed by the analysis of variance method (Snedecor and Cochran, 1980). Duncan's multiple range test was used to seperate treatment means (Duncan, 1955).

RESULTS & DISCUSSION

Cooking characteristics

The cooking times of field pea, moth bean and pigeon pea were 60, 45, and 90 min, respectively, and were comparable to those of other legumes (Meiners et at., 1976). On dehulling, the cooking times decreased from 60 to 35 min, from 45 to 20 min and from 90 to 30 min, respectively, for the three legumes. On cooking, field pea and moth bean absorbed about the same amount of moisture while pigeon pea absorbed relatively more moisture. Subsequently, the ratio of cooked weight to dry weight for pigeon pea was considerably higher (2.43) than that of moth bean (2.20) and field pea (2.14). The ratio of cooked volume to dry volume showed a similar trend with a value of 2.79 for pigeon pea, 2.50 for moth bean and 2.22 for field pea. The cooked weight/dry weight as well as cooked volume/dry volume ratios tended to be slightly higher for the dehulled (dhal) samples. These values were 2.51 and 2.95, 2.30 and 2.55, and 2.18 and 2.48 for pigeon pea, moth bean and field pea, respectively. Legumes showing higher yields on cooking i.e. higher values for cooked/uncooked legumes, are more acceptable with consumers (Manimekalai et al., 1979).

Proximate analyses

Data on proximate composition and energy values are given in Table 1. The moisture content of the whole seed legumes was in good agreement with literature values (Watt and Merrill 1963; Kuzayli et al., 1966; Kadwe et al., 1974). The protein content of field pea was higher than that of moth beans and pigeon pea. The values for protein contents were in agreement with literature values (Watt and Merrill, 1963; Kadwe et al., 1974). The data for the remaining proximate principles also agreed with the values reported in the literature for these legumes (Meshram et al., 1980; Shrivastava and Bajpai 1980; Deosthale and Sankar Rao, 1981), except the fiber content of field pea (8.1%) which was higher than the literature values

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Table 1—Proximate Composition[a] of Raw and Cooked Mature Dry Legumes and Water drained after cooking

Legume	Physical state	Moisture %	Protein (N × 6.25) %	Fat %	Ash %	Crude fiber %	NFE[b,d] %	Total[d] carbohydrates %	Energy[c,d]/100 g kcals	Energy[c,d]/100 g MJ
Raw										
Field pea	Seeds	8.5 ± 0.4b	24.5 ± 0.9g	1.4 ± 0.02d	3.0 ± 0.04e	8.1 ± 0.19f	54.5	62.6	329	1.38
	Dhal	7.6 ± 0.3ab	26.6 ± 0.4i	1.9 ± 0.02e	2.7 ± 0.01d	3.2 ± 0.11c	58.0	61.2	356	1.49
Moth bean	Seeds	10.0 ± 0.2c	22.7 ± 0.7f	1.0 ± 0.01bc	3.5 ± 0.04f	5.1 ± 0.09e	57.7	62.8	331	1.38
	Dhal	11.0 ± 0.3c	25.3 ± 0.3h	1.3 ± 0.02cd	3.0 ± 0.03e	2.0 ± 0.10b	57.4	59.4	343	1.44
Pigeon pea	Seeds	10.0 ± 0.3c	21.3 ± 0.6e	1.2 ± 0.01cd	4.5 ± 0.01h	8.2 ± 0.17f	54.8	63.0	315	1.32
	Dhal	6.8 ± 0.2a	25.6 ± 0.2h	1.9 ± 0.01e	4.0 ± 0.02g	3.0 ± 0.14c	58.7	61.7	354	1.48
Cooked										
Field pea	Seeds	60.2 ± 0.1d	10.9 ± 0.3d	0.7 ± 0.01ab	1.1 ± 0.02b	4.0 ± 0.13d	23.1	27.1	142	0.59
	Dhal	65.3 ± 1.2e	10.8 ± 0.3d	0.8 ± 0.01ab	0.8 ± 0.01a	1.8 ± 0.11b	20.5	22.3	132	0.55
Moth bean	Seeds	63.6 ± 0.9e	10.2 ± 0.3cd	0.5 ± 0.01a	1.3 ± 0.01bc	2.9 ± 0.11c	21.5	24.4	131	0.55
	Dhal	68.7 ± 2.4f	9.5 ± 0.2bc	0.5 ± 0.01a	0.8 ± 0.02a	1.1 ± 0.12a	19.4	20.5	120	0.50
Pigeon pea	Seeds	64.9 ± 0.9e	8.5 ± 0.4a	0.5 ± 0.08a	1.5 ± 0.04c	4.3 ± 0.16d	20.3	24.6	121	0.51
	Dhal	68.5 ± 1.9f	9.2 ± 0.2ab	0.8 ± 0.02ab	1.1 ± 0.02b	1.3 ± 0.14a	19.1	20.4	120	0.50
Cooking water[d]										
Field pea	Seeds	99.1 ± 0.2	0.26 ± 0.1	0.01 ± 0.00	0.21 ± 0.04	—	—	0.42	—	—
	Dhal	98.7 ± 0.2	0.45 ± 0.1	0.01 ± 0.00	0.34 ± 0.08	—	—	0.50	—	—
Moth bean	Seeds	98.7 ± 0.3	0.29 ± 0.2	0.01 ± 0.00	0.11 ± 0.03	—	—	0.89	—	—
	Dhal	98.5 ± 0.2	0.45 ± 0.1	0.01 ± 0.00	0.16 ± 0.02	—	—	0.88	—	—
Pigeon pea	Seeds	98.5 ± 0.2	0.26 ± 0.1	0.01 ± 0.00	0.09 ± 0.01	—	—	1.14	—	—
	Dhal	98.1 ± 0.3	0.57 ± 0.1	0.01 ± 0.00	0.28 ± 0.05	—	—	1.04	—	—

[a] Results are average of two lots determined in triplicate (n = 6) ± Standard Deviation where necessary. Means in a column followed by different letters are significantly different ($P \leq 0.05$).
[b] NFE is Nitrogen-Free Extract
[c] Calculated values. MJ is megajoule.
[d] Significance of differences not determined.

Table 2—True retention[a] values (%) of the three legumes on cooking

Nutrient	Field pea		Moth bean		Pigeon pea		Average		CV[b]%	
	Whole seed	Dhal	Whole seed	Dhal	Whole seed	Dhal	Whole seed	Dhal	Whole seed	Dhal
Protein	95c	89b	99d	86a	97cd	90b	97	88	2.1	2.4
Fat	107d	92ab	110d	89a	101c	93b	106	91	4.8	2.3
Ash	78d	65b	82e	61a	81e	69c	80	65	2.7	6.2
Fiber	106a	123b	124b	127c	128c	109a	119	120	9.8	7.9
NFE	91c	77a	82b	78a	90c	82b	88	79	5.6	3.3
Energy	92c	81a	87b	81a	93c	83a	91	82	3.6	1.5

[a] True retention (mean,%) calculated by the formula of Murphy et al. (1975). Means (%) in the horizontal column followed by different letters are significantly different ($P \leq 0.05$).
[b] CV is coefficient of variation

for dry mature pea seeds (Kuzayli et al., 1966; Watt and Merrill, 1963). The energy values of the three legumes were compatible with the data on other legumes (Meiners et al., 1976).

As expected, the moisture content of the cooked legumes was greater than in the raw legumes. The increase in moisture content had a dilution effect on all other nutrients in the cooked legumes. The values for protein, fat, ash, crude fiber, NFE as well as energy contents of the cooked samples were comparable to the data of Meiners et al. (1976). The analysis of cooking water (Table 1) indicated that carbohydrates leached the most into the cooking water, followed by proteins and ash. When the leached solids were expressed as a percentage of the legumes cooked, the loss of total solids was 10.2% for field pea, 12.7% for moth bean and 14.9% for pigeon pea. Dehulling increased the protein, fat and energy contents but decreased the ash and fiber contents. On cooking, dhal samples showed a higher content of moisture but lower contents of ash, carbohydrates and crude fiber. The cooking water (Table 1) from dhal samples contained higher amounts of dry matter compared to whole seeds as indicated by the lower moisture content of cooking water from dhal samples. Consequently, slightly higher losses of dry matter occurred in dhals which, when expressed as a percentage of dhal cooked, were 12.0% for field pea, 13.9% for moth bean and 19.0% for pigeon pea.

True Retentions

Data on true retentions of nutrients after cooking are shown in Table 2. The maximum retention was observed for crude fiber and the minimum for ash in both the seeds and dhal. Dhals showed significantly ($P \leq 0.05$) lower retention values compared to the whole-seeds mainly due to the higher loss of solids during cooking. The average retention values for the seeds were in reasonably good agreement with the data of Murphy et al. (1975).

In conclusion, these legumes were comparable to the commonly used legume seeds in proximate composition, cooking behavior and retention of proximate principles on cooking. Although substantial nutrient data are available on raw legumes and some of their products, the effect of cooking on the nutrient density in cooked legumes is rare except for the work of Meiners et al. (1976). The present results would, therefore, augment the scarce information on cooked legumes. Moreover, data on weight-volume relationship would be useful in predicting the concentration of nutrients in the cooked legumes from the data on raw legumes and to calculate appropriate retention factors.

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A Research Note

Nonfat Dairy Coffee Whitener Made from Ultrafiltered Skimmilk Retentates

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ABSTRACT

Retentates of different protein concentrations obtained by ultrafiltration of skimmilk were freeze-dried and evaluated as nonfat dairy coffee whiteners. Blended in hot coffee the retentate whiteners containing added riboflavin gave a pH of 6.3–6.55, coffee whitening capacity comparable to a commercial nondairy coffee creamer, and acceptable dispersibility. The retentate nondairy whitener with optimum qualities contained 56% total protein, 0.5% fat, 31.0% carbohydrates, 1.92% calcium, and 27 mg sodium/100g.

INTRODUCTION

NONDAIRY COFFEE CREAMERS (NDCC) are composed largely of corn syrup solids and vegetable fats, according to Lampert (1970). Posati and Orr (1976) and Jolly and Kosikowski (1978) report that a very high percentage of the fatty acids in commercial NDCC are saturated.

Ultrafiltration (UF) can produce dried skimmilk retentates that display different composition and physical properties and improved nutritional value compared to standard skimmilk powders (Jimenez-Flores, 1984; Jimenez-Flores and Kosikowski, 1985). The objective of the present study was to assess the potential of ultrafiltered skimmilk retentate powders as nonfat dairy coffee whiteners.

MATERIALS & METHODS

FOR EACH TRIAL 320 liters of freshly drawn, raw Holstein milk from the Cornell University Veterinary farm were heated to 54°C. Three-quarters of this milk volume was ultrafiltered in an Abcor 22S UF unit with 2 m^2 of polysulfone high flux membranes, possessing a molecular weight cut-off of 20,000 daltons. Inlet and outlet pressures were 310.3 and 103.4 kPa, respectively. Three retentate lots at 2:1, 3:1, and 4:1 volume concentration were produced. A fourth lot, that of the heated raw milk, serving as a control, was concentrated to 20% total solids (T.S.) in an APV falling film plate evaporator (model J.P.W.). Condensed milk control and the retentates then were mechanically separated in a De Laval (Model 242) separator and pasteurized at 72°C and 15 sec. These lots were freeze-dried in a Virtis UE 800 unit and resulting flakes were mechanically milled. Powder A was a control from condensed skimmilk. Powders B, C, and D were from skimmilk retentates ultrafiltered to 2:1, 3:1 and 4:1 volume concentration. For comparative purposes commercial NDCC were obtained as well as a commercial low-heat skimmilk powder.

Riboflavin (Eastman Kodak reagent) and beta-carotene (Sigma Chemicals) were added to retentate nonfat dairy whiteners at 10 mg/100g powder to enhance color.

Analysis

A HunterLab Color Difference-meter, Model D25, (Hunter Associates Laboratory, Fairfax, VA) was used to quantitate whitening capacity and color differences in 80°C coffee of nonfat retentate dairy whiteners and a NDCC. Two grams powder were dissolved in 250 mL of a 'standard coffee solution', (4g Maxwell House instant coffee in 500 mL hot water). Color analyses made in triplicate are described elsewhere with more detail (Jimenez-Flores, 1984). Also, color photographs were taken of the coffees.

Dispersibility of the powders was measured in 24°C water as percentage values by the method of Baldwin (1977) and in hot coffee visually by the authors. The pH of the whitened coffee was determined by a Beckman Expandomatic potentiometer.

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Table 1—Properties of individual skimmilk retentate powders

	Freeze-dried powders[a]				Nondairy coffee creamer powder
	A	B	C	D	Y
pH in hot coffee[b]	6.00	6.30	6.45	6.55	6.30
Total color difference in hot coffee	6.52	5.48	4.37	3.40	7.20
Percent dispersability in water[c]	34	32	32	33	100

[a] A produced from laboratory skimmilk powder control, B from 2:1 UF retentate, C from 3:1 UF retentate, D from 4:1 UF retentate, Y = national brand.

[b] Fresh unwhitened coffee pH = 5.30.

[c] Commercial low heat skimmilk powder displayed a dispersability value of 37.

RESULTS

TOTAL COLOR DIFFERENCE, pH and dispersibility of the five experimental powders are shown (Table 1). Nonfat dairy whiteners, identified as skimmilk retentate powders, adding to hot, black coffee (pH 5.3) shifted the pH from 6.3 to 6.55, equalling, or slightly exceeding, the pH when the same black coffee was colored by a NDCC. Color difference ($\triangle E$) of hot coffee containing nonfat dairy whiteners compared against a white standard, were less than those for a commercial skimmilk powder control and a NDCC. Value for NDCC dispersibility in water was 100% and for nonfat dairy whiteners 32–34%. Powder 1, and commercial low heat skimmilk powder, both controls, showed dispersibility values between 34–39%. In hot coffee NDCC was observed to disperse immediately whereas nonfat dairy coffee whiteners dispersed more slowly but uniformly.

The whitening capacity and color difference of only UF skimmilk retentate powders C and D in hot coffee are presented in Table 2. These powders were selected because of their higher nutritional qualities. Small additions of riboflavin in particular enhanced color quality and equalized to a great extent color differences, eliminating a greyish cast which was apparent visually.

Color photographs, not shown, record that a commercial low heat skimmilk powder compared to retentate nonfat dairy whiteners or to a NDCC appears deficient in hot coffee whitening power. Conversely nonfat dairy whiteners C and D containing added riboflavin show good whitening power in hot coffee, equal to that of a NDCC.

DISCUSSION

NONFAT DAIRY COFFEE WHITENER C contained 56.4% protein, 0.5% fat, 31.4% carbohydrate, 1.9% calcium, and 27.5 mg sodium per 100g. By contrast Posati and Orr (1976)

Table 2—Whitening capacity nonfat dairy whiteners C and D in hot coffee

Whitener powder[e]	L[a]	A[a]	B[a]	DL[b]	Da	Db	DE	dL[c]	da	db	dE
C	46.9 (1.2)	3.7 (0.1)	15.3 (0.9)	46.2	4.5	25.9	49.06	1.4	0	3.5	3.77
C + R + Beta	47.3 (1.3)	3.7 (0.1)	15.7 (0.8)	45.8	4.5	16.3	48.5	1.0	0	3.1	3.26
C + R	47.1 (1.2)	3.5 (0.1)	26.0 (1.0)	46.0	4.3	16.6	49.1	1.2	0.2	2.8	3.05
C + Beta	47.3 (1.2)	3.7 (0.1)	15.4 (0.9)	45.8	4.5	16.0	48.7	1.0	0	3.4	3.54
D	48.55 (1.3)	3.3 (0.1)	14.6 (1.1)	44.6	4.1	15.2	47.3	0.25	0.2	4.2	4.2
D + R + Beta	48.9 (1.2)	3.1 (0.1)	15.4 (1.0)	48.6	3.9	16.0	47.2	−0.6	0.6	3.4	3.5
D + R	49.0 (1.2)	2.8 (0.1)	15.3 (0.1)	44.1	3.6	15.9	47.0	−0.7	0.9	3.5	3.5
D + Beta	48.8 (1.4)	3.2 (0.1)	14.8 (1.2)	44.3	4.0	15.4	47.1	−0.5	0.5	4.0	4.1
White Standard	93.1	−0.8	−0.6	0	0	0	—	—	—	—	—
NDCC	48.3	3.7	18.8	44.8	4.5	19.4	49.03	0	0	0	0

[a] Arithmetic mean of 2 determinations for 4 samples, values in ( ) are standard deviations. L = Lightness, 100 perfect white, 0 perfect black; A = redness; B = yellowness.
[b] D — (columns 5–8) indicates that the difference has been taken from the white standard.
[c] d — (columns 9–12) indicates that the difference is with respect to the NDCC.
[d] a = Redness when +, greeness when −, gray when 0; b = Yellowness when +, blueness when −, gray when 0; E = Total color difference.
[e] Powders C and D were 3:1 and 4:1 skimmilk retentates, respectively. NDCC = Nondairy coffee creamer; R and Beta (column 1) = riboflavin and beta-carotene.

list NDCC powders on the average as containing 4.8% protein, 35.5% fat, 54.9% carbohydrate, 0.02% calcium and 181 mg sodium per 100g. Nonfat dairy coffee whitener C is preferred over whitener D because greater opportunity exists for sedimentation at protein levels above 60% as observed visually and because it is less expensive to produce.

Although NDCC powders dispersed into hot coffee immediately, under the same conditions, retentate nonfat dairy whitener C and D dispersed satisfactorily after one spoon rotation. Spray dried, nonfat dairy whiteners were also produced in the laboratory from the same liquid retentates. Dispersibility of the higher protein spray-dried retentates was not fully satisfactory, a condition which may be corrected by instantizing or agglomerating the powder and attaining better control over variables during commercial spray drying.

Three additional commercial NDCC powders obtained for more detailed studies involving functionality, influenced whitening of hot coffee to approximately the same degree as the NDCC used specifically for the coffee creamer study. NDCC whitening effects are mainly achieved from fat and artificial colors while those of retentate nonfat dairy whiteners are obtained through milk protein and added riboflavin or beta-carotene. Such whiteners may have the potential also for use in concentrated liquid form, perhaps aseptically packaged.

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COOKING EFFECT ON LEGUME YIELD/COMPOSITION. . .

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A Research Note

Investigation of Cashew Apple Juice as a Substrate for Single Cell Protein Production

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ABSTRACT

Cashew juice was investigated for its ability to support the growth of *Saccharomyces cerevisiae*. A characteristic of the juice showed that it contained a mixture of fermentable sugars of which glucose, fructose and sucrose have been identified. Also the concentration of reducing sugars was as high as 15% w/v. Growth studies were carried out in a 7-L batch fermentor at a pH of 5.0 and 30°C with and without nutrient supplementation. The corresponding yields were 0.50 and 0.39 gram cells/gram reducing sugar consumed, respectively. While about 10% of the reducing sugar was consumed in the unsupplemented medium 43% was consumed in the medium with supplementation.

INTRODUCTION

THE NEED for single cell protein (SCP) cannot be over emphasized. An acute shortage in meat and fish meals has brought man and animal into competition for cereals which are the stable foods in many developing countries. Growing demand for meat has caused some meat-exporting countries to rely more heavily on grain staples produced internally as components of their animal feeds. In the face of uncertain crop yields and the ever-increasing world demand for meat products, the feed industry is obviously attracted by a product of constant composition with good nutritional value which can be produced under easily controlled conditions. The SCP is a good example of such a product.

The microbial proteins may be used to supplement human and animal diets in the wake of the sweeping starvation and malnutrition problems facing many developing countries (Reese et al., 1972; Bellamy, 1969; Ghose, 1977). The cashew plant (*Anacardium occidentale*), is highly drought-resistant, grows well on poor soils and has high productivity. Although cashew trees are spread throughout the tropics, commercial production is centered in India which handles most of the world trade. Usually the fruits fall from the trees when mature and are gathered by local labor. The nuts, which have hitherto been the primary products of interest, are separated from the apples which are generally wasted. Such wastes may be used in microbial processes (Lemmel et al., 1979; Moreton, 1978; Bloch et al, 1973; Jarl, 1969; Solomon et al, 1981; Layokun, 1984).

Earlier works by Swabey (1975) and Falade (1981) reported the total reducing sugar in cashew juice but no attempts were made to identify the sugar types. Hence in this work, the characterisation of the apple juice to identify the sugar types is carried out in addition to investigating its ability to support the aerobic growth of *S. cerevisiae* in batch systems.

MATERIALS & METHODS

A BENCH-SCALE FERMENTOR, model 19, produced by New Brunswick Scientific Co. was used. The cells were cultivated in a 7-L stirred container under aseptic conditions. Temperature was maintained at 30°C with a temperature controller, and the pH was held at 5.0 by a New Brunswick Scientific model pH-22 controller which added 1N NaOH or 1N H_2SO_4 through two peristaltic pumps. Foaming was controlled both chemically and mechanically. Chemical control was achieved by adding a few drops of sterilized palm oil to the medium.

The cashew fruits were collected from a farm in Isanlu area of Kwara State, Nigeria. The apples were pulped using the Stephen Universal Machine Type UMB 25. The juice extracted with a Genzang orangematic hand press filter, was weighed and sterilized in an autoclave for 15 min at 15 psi steam pressure (121°C) and later stored at 4°C.

Saccharomyces cerevisiae NCYC 1250 was used throughout this study. The innoculum was prepared by adding 2g of the freeze-dried organism to 50 mL sterilized water. Ten milliliters of the homogenized mixture was pipetted aseptically into 100 mL yeast extract broth in 250 mL conical flasks. (The yeast extract broth was prepared by mixing 5g NaCl and 10g yeast extract in 1-L sterilized distilled water). The inoculated flasks were then transferred into the gyrotary incubator shaker where growth was achieved by operating at a temperature of 30°C and a shaker agitation rate of 200 rpm for 18 hr.

Aerobic fermentations were carried out in unsupplemented and supplemented media. The unsupplemented medium was prepared by diluting the original cashew apple juice to give a reducing sugar concentration of 30 g/L. However, the supplemented medium, which was prepared as a result of evident deficiency in the unsupplemented medium during initial studies, contained additional mineral salts as supplements. The composition of the supplemented medium was similar to that used by Solomon et al. (1981) for the growth of *Candida utilis* onhydrolyzed grain dust. Although three runs were carried out with each medium, only a typical set of results is presented in Fig. 1.

For each run, the fermentor vessel containing about 4L sterilized medium was inoculated with the incubated innoculum. Also for all runs, the agitation rates were set at 800 rpm, while aeration was maintained at 1 vvm (volume of air per volume of broth per minute). Samples were withdrawn at suitable intervals and analyzed for reducing sugar and biomass concentrations.

Analytical methods

Cell dry weight was determined as described earlier by Layokun (1984). The reducing sugar concentrations in the initial cashew juice and in the samples withdrawn during fermentation were determined using modified dinitrosalicylic acid (DNS) reagent (Miller, 1959).

Qualitative analysis of the sugar content of cashew juice was carried out by thin-layer/paper chromatography following the procedure described by Stahl (1969).

RESULTS & DISCUSSION

ONE OF THE MOST IMPORTANT factors which determine the suitability of a particular substrate for SCP production is the nature of the sugars in it. Thus, characterization of the simple sugars in the cashew apple juice was initially carried out. Both the thin-layer and paper chromatographic examinations revealed the presence of sucrose, glucose, and fructose. Another important factor to consider in substrate evaluation for SCP production is the total amount of fermentable sugars. The extraction method employed in this work, extracted 79% of the cashew apple as juice. An analysis of this juice showed that it contained 14.84% w/v reducing sugars. This is in good agreement with the 15% w/v value reported by Falade (1981) as opposed to the 7–9% w/v reported by Swabey (1975). This high value of total reducing sugar is desirable in SCP produc-

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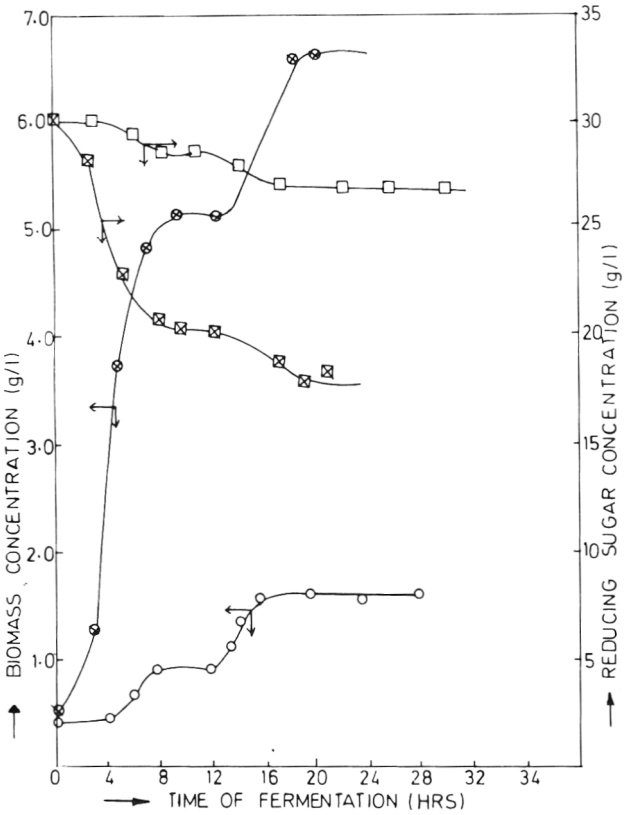

Fig. 1 — Plot of microbial biomass concentration and reducing sugar concentration against fermentation time for growth on unsupplemented and supplemented cashew juice: □, reducing sugar concentration for unsupplemented medium; ⊠, reducing sugar concentration for supplemented medium; ○, Biomass concentration for unsupplemented medium; ⊗, Biomass concentration for supplemented medium.

tion since it allows for a high productivity which is necessary for an economical process.

Fig. 1 contains a plot of cell and reducing sugar concentrations as a function of time for the aerobic growth of *Saccharomyces cerevisiae* on the unsupplemented and supplemented cashew juice. This figure reflects a behavior of diauxic growth in both cases. This is expected since the TLC/PC characterization showed the presence of a mixture of sugars in the fresh juice. Also, the paper chromatographic analysis of samples withdrawn from separate shake flask experiments using supplemented medium showed that glucose was consumed first while the sucrose was being hydrolyzed. This led to the accumulation of fructose which was later consumed. Thus the first logarithmic phase corresponds to glucose consumption while the second corresponds to fructose consumption.

Also from Fig. 1 it is observed that for the unsupplemented medium only 3.0 g/L of the starting 30 g/L reducing sugar was used even after prolonged fermentation time. This may be explained by the presence of very low concentrations of essential nutrients. Thus the experiment was repeated with nutrient supplementation. The results show that about 13 g/L of the sugar was now consumed (Fig. 1). It can also be observed that the rate of growth increased significantly while the initial lag was shortened considerably.

Analysis of both the unsupplemented and supplemented growth studies revealed that growth yields of about 0.39 and 0.50g microbial biomass/g consumed reducing sugar were obtained, respectively, under the two conditions. Thus the growth in the unsupplemented medium, which was probably under nitrogen limitation, required that a lot of the substrate was consumed just to maintain the cells; however, with supplementation, the maintenance requirement had been significantly decreased (Solomon et al., 1982).

CONCLUSIONS

BASED ON the level of sugar in the cashew juice and the ability of *Saccharomyces cerevisiae* (a yeast with proteins which are of good nutritional quality (Sandhu and Waraich, 1983) and contains about 50% crude protein (Solomon et al., 1981) to utilize them, cashew juice may be converted to a high protein product when supplemented with essential mineral salts. The results of this work should be useful in evaluating the potential of cashew juice as a feedstock for SCP production.

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A Research Note

Influence of Storage Duration and Temperature on Sweet Potato Sugar Content and Chip Color

DAVID H. PICHA

ABSTRACT

Sugar concentration and chip color of three orange-flesh and two white-flesh sweet potato cultivars were determined after various lengths of storage at different temperatures. Lightest colored chips were produced from roots chipped immediately after harvest and from cultivars which contained the lowest levels of glucose and fructose. Storage of roots at 32°, 15.6°, or 7°C after harvest resulted in increased sucrose, glucose and fructose concentrations and darker colored chips. Manipulation of storage temperature was not successful in lowering reducing sugar concentration in any cultivar.

INTRODUCTION

SWEET POTATO CHIPS were initiated as a consumer product in 1936 (Brunstetter, 1936). Although production techniques have been refined so that sweet potato chips are a commercial possibility (Sistrunk and Miller, 1954; Burton et al., 1958; Kelley et al., 1958; Boggess and Woodroof, 1964; Hoover and Miller, 1973), only limited local production of sweet potato chips exists. A major consideration in the establishment of a successful sweet potato chip industry is the year round availability of roots which will process into desirable colored chips of good flavor, texture, and low oil content.

Since the U.S. sweet potato crop is usually harvested from August through November, it is necessary to store roots over an 8-month period to supply chip processors. Sugar content in sweet potatoes differs between cultivars (Picha, 1985a) and length of storage (Walter and Hoover, 1984) which may influence chip quality. The purpose of this study was to determine the effect of cultivar, storage duration and temperature on sugar content and chip color of sweet potatoes.

MATERIALS AND METHODS

TWO WHITE-FLESH (Whitestar, Rojo Blanco) and three orange-flesh (Centennial, Jewel, Travis) sweet potato [*Ipomoea batatas* (L.) Lam] cultivars were grown in Baton Rouge, LA following commercially recommended practices (Montelaro et al., 1966). Four replications of six No. 1 grade roots were analyzed the day of harvest and after different periods of storage at different temperatures. Relative humidity was 85–90% in all storage rooms.

Individual sugars in the raw roots were extracted and their concentrations were determined by HPLC as previously described (Picha, 1985a).

Washed, untreated roots were cut 1.5 mm thick with a power-driven vegetable slicer, rinsed in cold running water to remove surface starch, and drained of excess water before frying. Slices were fried in vegetable oil at 163°C for 1.5 min, which coincided with the cessation of bubbling. Temperature reduction upon insertion of the slices into the oil bath of the stainless steel deep-fat fryer was negligible. A commercially available vegetable oil was used because of its stability and failure to harden at room temperature on the surface of the chips. Chip color ratings were based on the Potato Chip Institute International Color Standard. The white-flesh cultivar rating scale ranged from 1 (light white) to 10 (dark brown) and the orange-flesh cultivar rating scale ranged from 5 (light orange) to 10 (dark brown).

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RESULTS & DISCUSSION

CHIPS made from Centennial and Jewel the day of harvest were an attractive bright orange in appearance, contained the lowest amount of sugars and produced the lightest colored chips (Table 1). Chip color was darker in both cultivars after the roots were cured. Jewel chips continued to darken to an orange-brown color during the first 6 wk of storage at 15.6°C, but no further darkening occurred from 6 to 44 wk. Centennial chips gradually darkened over the first 12 wk, after which no further darkening was apparent. Chip color in uncured Centennial and Jewel was as dark after 2 wk at 15.6° or 7° as in cured roots stored for 2 wk at 15.6°C. Two weeks at low temperature (7°C) followed by 2 wk at high temperature (32°C), or the reciprocal treatment, was not successful in lowering the sugar concentration or producing a similar light chip as at harvest. Since manipulation of postharvest temperature did not prevent sugar accumulation or chip darkening, roots intended for chip production can be stored under the same recommended conditions (cured + 15.6°C) as those used for table stock. Centennial chips were lighter than Jewel at all times except at harvest. This cultivar difference and storage treatment effect was associated with a higher glucose and fructose concentration in Jewel than Centennial and increased reducing sugar levels with storage. Sucrose also increased during curing but was not directly responsible for darkening since the highest sucrose containing cultivar (Centennial) produced the lighter colored chips.

In addition to lighter chip color, Centennial roots also have a higher total carotenoid content, more crude protein, and higher dry matter than Jewel which would result in a greater yield of chips from Centennial (Picha, 1985b).

High reducing sugars combined with amino acids through a Maillard reaction are the cause of darkening in Irish potato chips (Habib and Brown, 1957). This sugar buildup occurs most rapidly at temperatures 4°C or lower (Schwimmer et al., 1954; Hawkins et al., 1958; Paez and Hultin, 1970). Irish potatoes can be reconditioned by high temperature (21°C) storage for several weeks to lower the sugar content and produce light chips. A similar mechanism of sugar transformation apparently does not exist in sweet potatoes, which are chilling injury susceptible and subject to abnormal metabolism when stored below 12°C (Picha, 1984). Sugars continued to accumulate in sweet potatoes after harvest regardless of whether the roots were stored at 7°, 15.6° or 32°C.

Chips from Travis roots were much darker than chips from Centennial or Jewel roots. Travis roots chipped the day of harvest had a color rating of 8 and 0.51% glucose and 0.43% fructose. Chip color further darkened after curing and storage which was associated with total reducing sugar levels in excess of 2%. Blanching raw slices from Travis roots in sodium acid pyrophosphate followed by partial dehydration before frying may produce a lighter chip (Hoover and Miller, 1973), which may be of further value in minimizing discoloration in Centennial and Jewel.

Chips made from the two white-flesh cultivars the day of harvest were very light, but color darkened after curing and was even darker after 6 to 44 wk storage at 15.6°C (Table 2). Whitestar chips were always lighter than Rojo Blanco after the

Table 1—Chip color rating and sugar concentration in Centennial (Cent) and Jewel (Jwl) sweet potatoes after different storage treatments

Treatment	Chip color rating[ab]		% Sugar (fresh weight basis)					
			Glucose[b]		Fructose[b]		Sucrose[b]	
	Cent	Jwl	Cent	Jwl	Cent	Jwl	Cent	Jwl
At Harvest	5	5	0.04	0.09	0.07	0.07	1.80	1.72
Cured[c](C)	6	7	0.08	0.61	0.17	0.42	3.11	2.64
C + 2 wk 15.6°C	6	7	0.12	0.70	0.16	0.46	3.21	2.61
C + 6 wk 15.6°C	6	8	0.15	1.02	0.17	0.88	3.78	2.68
C + 12 wk 15.6°C	7	8	0.24	1.22	0.30	1.01	4.10	2.78
C + 20 wk 15.6°C	7	8	0.35	1.25	0.39	1.05	4.40	3.36
C + 44 wk 15.6°C	7	8	0.46	1.27	0.40	1.00	5.15	4.09
2 wk 15.6°C	6	7	0.13	0.53	0.12	0.50	3.02	2.34
2 wk 7°C	6	7	0.10	0.40	0.09	0.42	3.20	2.93
2 wk 7°C + 2 wk 32°C	7	8	NA[d]	NA	NA	NA	NA	NA
2 wk 32°C + 2 wk 7°C	6	8	0.14	0.89	0.13	0.58	4.50	3.73

[a] Rating scale from 5 (light orange) to 10 (dark brown).
[b] Average of 4 replications.
[c] Cured (C) = 10 days at 32°C, 90% RH.
[d] Samples not analyzed for sugar content.

Table 2—Chip color rating and sugar concentration in Whitestar (WS) and Rojo Blanco (RB) sweet potatoes after different storage treatments

Treatment	Chip color rating[ab]		% Sugar (fresh weight basis)					
			Glucose[b]		Fructose[b]		Sucrose[b]	
	WS	RB	WS	RB	WS	RB	WS	RB
At Harvest	2	3	0.08	0.11	0.09	0.11	1.14	0.81
Cured[c] (C)	4	6	0.27	0.69	0.23	0.42	2.99	1.98
C + 2 wk 15.6°C	4	6	0.24	0.66	0.25	0.32	2.50	1.60
C + 6 wk 15.6°C	5	7	0.34	0.85	0.32	0.55	2.81	1.46
C + 12 wk 15.6°C	5	7	0.40	0.95	0.39	0.65	2.50	1.30
C + 20 wk 15.6°C	6	7	0.50	1.03	0.50	0.65	2.41	1.43
C + 44 wk 15.6°C	6	7	0.56	0.89	0.49	0.55	2.45	2.32
2 wk 15.6°C	5	6	0.33	0.69	0.37	0.70	2.42	1.26
2 wk 7°C	5	6	0.36	NA[d]	0.46	NA	4.66	NA
2 wk 7°C + 2 wk 32°C	5	7	NA	NA	NA	NA	NA	NA
2 wk 32°C + 2 wk 7°C	5	7	0.34	NA	0.33	NA	5.27	NA

[a] Rating scale from 1 (light white) to 10 (dark brown).
[b] Average of 4 replications.
[c] Cured (C) = 10 days at 32°C, 90% RH.
[d] Samples not analyzed for sugar content.

same storage treatment. This cultivar difference and storage treatment effect was again associated with higher concentrations of glucose and fructose in Rojo Blanco than in Whitestar and increased reducing sugar levels during storage. There was a lack of association between chip darkening and sucrose level. Various combinations of low and high temperature storage treatments were unable to prevent chip darkening.

Consumer acceptability of Whitestar and Rojo Blanco chips is questionable based on Irish potato standards. Hawkins et al. (1958) found it was not possible to make acceptably light colored Irish potato chips if the concentration of reducing sugars was above 0.4%. However, Porter et al. (1973) were able to produce acceptable colored chips when reducing sugars were 0.9% or below by using a procedure of partial oil-frying followed by microwave finishing. This technique may have applicability with Whitestar roots if storage length is 12 wk or less.

Additional observations revealed no difference in chip color between apical or basal slices from any cultivar. The inner storage parenchyma tissue beneath the cambium was darker than the cambium, cortex and periderm tissues. Less folding of slices during frying occurred with smaller diameter roots. Optimum frying temperature was 163°C. Higher oil temperatures produced darker chips, as previously reported (Hoover and Miller, 1973). Lower oil temperatures lengthened the frying time required but did not produce lighter chips. Chips of all cultivars absorbed high amounts of oil. Less oil absorption occurs with decreasing moisture content of the unfried slices (Hoover and Miller, 1973). Additional research is needed to minimize sweet potato chip oil content by testing different types of oil and utilizing different finishing techniques such as microwave drying.

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A Research Note

Protein Nutritive Value of Hinoat and Scott Oat Cultivars and Concentrates

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ABSTRACT

Protein sources studied were oat groats, oat crude protein extracted at pH 9.5 (PE) and oat protein concentrate obtained by isoelectric precipitation (PC) of the alkaline extract. PER of Scott proteins was higher than that of corresponding Hinoat proteins: 2.31 ± 0.17 vs 2.15 ± 0.12 for groats, 2.08 ± 0.13 vs 1.93 ± 0.11 for PE and 2.11 ± 0.12 vs 1.97 ± 0.06 for PC. However, for both cultivars, PERs of PC and PE were lower than PER for groats. There was no difference between NPR of Scott and Hinoat for the same protein fractions. Apparent digestibility coefficient (ADC) of Scott proteins were lower than the corresponding Hinoat proteins, and for both cultivars protein ADCs were in the following order: PC > PE > groats.

INTRODUCTION

OATS (*Avena sativa* L.) is the fifth largest cereal crop in the world. Oat groats generally rank higher in protein content (10–22%) and in potential for protein production than other cereal crops (Burrows, 1983). The nutritive value of oat protein is higher than other cereal grains (Robert et al., 1983), and proteins extracted from soybean (Kjaergaard and Bruzelius, 1980). In spite of its high nutritive value, most oats are utilized as animal feed (Youngs et al., 1982). In Canada, between 1982 and 1983, only 2.1% of the oat crop was used for human consumption (Statistics Canada, 1984).

Oat protein concentrates or isolates with a protein content varying between 48 and 99% (Wu and Stringfellow, 1973; Wu et al., 1977; Kjaergaard and Bruzelius, 1980; Ma, 1983a) have been prepared using various methods. However, information on these products has been limited to chemical composition and functional properties (Wu et al., 1977; Cluskey et al., 1978; Ma, 1983a, b). To our knowledge, there are no available data on the biological evaluation of their protein quality. This evaluation is however essential before using these concentrates or isolates in foods for humans.

The purpose of the present study was to measure the protein nutritive value of Hinoat and Scott oat cultivars and concentrates.

MATERIALS & METHODS

PROTEIN CONCENTRATES of Hinoat and Scott cultivars were prepared by alkali extraction at pH 9.5 using a solvent:groat ratio of 8:1 (Ma, 1983b). The slurry was first passed through a Comitrol grinder equipped with a microcut head (Urshel Laboratories Inc., Valparaiso, IN), stirred for 1 hr (pH 9.5) at room temperature and allowed to settle at 4°C. A crude protein extract (PE) and a residue were recovered. The residue was screened through a 100 mesh sieve to yield a starch and a bran fraction (BF). The PE was adjusted to pH 5.5 and clarified using a laboratory separator LAPX 202 (Alpha-Laval, Sweden) to yield a protein concentrate (PC) and a clear supernatant.

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Nutritional evaluation

Diets were formulated to contain 10% protein (N × 6.25) supplied as oat groats, PE and PC from both oat cultivars and as BF from Hinoat; it was not possible to formulate rations using Scott bran because its crude protein content was less than 10%. Casein was used as reference protein. Diet also contained 10% corn oil, 4% mineral mix (USP XVII, Teklad Test Diets, Madison, WI, USA) and 1% vitamin fortification mix (Teklad Test Diets). Corn starch was used to adjust digestible energy to 3.82 kcal/g (isocalorie), while cellulose (non nutritive fiber, Teklad Test Diets) used as a filler was given a zero value; the acid detergent fiber (ADF) fraction of protein sources was also given a zero value.

Net protein ratio (NPR) (Bender and Doell, 1957) and protein efficiency ratio (PER) were determined with Sprague-Dawley male rats weighing 57.4 ± 9.5g. They were allotted at random in groups of 10 rats each which were placed in individual cages and received food and water ad libitum. Feces were collected from day 3 to day 10 to determine the apparent digestibility coefficient (ADC) of proteins. Chromic oxide was added to diets at 0.1%.

Chemical analyses

Total nitrogen content of samples was measured by Kjeldahl method using a Kjel-Foss Automatic 16210 (A/S N. Foss Electric, Denmark). Crude energy was determined in a bomb calorimeter (Parr Instrument Co. Inc., Moline, IL). Acid detergent fiber (ADF) was measured according to the method of Goering and Van Soest (1970). Chromic oxide was measured according to the method of Christian and Coup (1954). Protein sources were hydrolyzed under nitrogen in 6N hydrochloric acid (Powder:acid ratio was 1:1000) at 110°C for 24 hr and amino acid content was determined using an amino acid analyzer (LKB Biochrom Ltd, Cambridge, England) equipped with an HP 3392A integrator.

Statistical analysis

Results were submitted to an analysis of variance (general linear models procedure) followed by Duncan's test (Little and Hills, 1978).

RESULTS & DISCUSSION

Nutritional evaluation

Groats. PER of Scott groats was higher ($p < 0.05$) than that of Hinoat groats (Table 1), and the values were respectively 80% and 74% of that of casein. NPRs were not significantly different between the two groats (Table 1). It is possible that a 10-day growing period was not long enough to discriminate between two proteins close in nutritional value. We have shown previously that PER was a method leading to more significant differences between a wide range of protein sources than NPR (Goulet et al., 1984). Effectively, in this experiment, the coefficient of variation for NPR and PER were 10.1% and 5.8% respectively (Table 1).

The protein ADC of Hinoat groats, was higher ($p < 0.05$) than that of Scott groats, (Table 1). This may be related to the protein content of Hinoat and Scott groats which were 22.8

Table 1—Feed intake, liverweight gain, NPR, PER and protein ADC[a] of rats fed experimental diets

Diet	Feed intake 0–10 days (g)	Feed intake 0–28 days (g)	Liveweight gain 0–10 days	Liveweight gain 0–28 days	NPR	PER	Protein ADC (%)
Hinoat:[e]							
groats	90.7a[b]	311.0bc	24.6b	70.3bc	3.49b	2.15c	83.2e
protein extract (PE)	82.2a	277.7c[c]	18.6c	58.9d[c]	2.98c	1.93e[c]	85.8c
protein conc (PC)	82.9a	293.9bc	20.0bc	62.1cd	3.29bc	1.97de	89.0b
bran fraction (BF)	65.1b[d]	—	11.7d	—	3.15bc	—	67.4g
Scott:[f]							
groats	92.9a	324.3b[c]	24.5b	76.2b[c]	3.39b	2.31b[c]	78.4f
protein extract (PE)	83.5a	308.1bc	20.8bc	69.3bcd	3.32bc	2.08cd	83.4e
protein conc (PC)	91.8a	323.6b[c]	23.0bc	74.2b[c]	3.24bc	2.11c[c]	84.7d
Casein (ANRC reference protein)	86.2a	380.0a	37.5a	123.6a	4.42a	2.90a	91.5a
C.V. %	13.6	11.4	23.8	14.7	10.1	5.8	1.5

[a] ADC - apparent digestiblity coefficient.
[b] Values in the same column bearing the same letter are not significantly different ($p > 0.05$).
[c] Mean values were calculated on nine rats.
[d] Results are available only for NPR.
[e] Hinoat groats were provided by Dr. V.D. Burrows.
[f] Scott seeds were purchased from Cribagro Inc. (Nicolet, Québec, Canada) and were dehulled before using.

Table 2—Amino acid composition (g/16g N)

Amino acid	Casein	Hinoat groats	Hinoat PE[a]	Hinoat PC[b]	Hinoat BF[c]	Scott groats	Scott PE[a]	Scott PC[b]
Essential								
Arginine	3.5	5.8	6.6	6.4	6.7	6.1	6.1	6.8
Histidine	2.6	2.0	2.1	2.2	2.1	2.0	2.1	2.2
Isoleucine	4.5	3.3	3.4	3.7	3.2	3.2	3.4	3.7
Leucine	7.7	6.0	6.4	6.6	5.8	6.0	6.2	6.6
Lysine	6.5	3.3	3.2	3.1	4.0	3.4	3.3	3.3
Methionine	2.6	1.0	1.3	1.4	1.0	0.8	1.2	1.3
Phenylalanine	4.6	4.4	4.7	5.1	4.1	4.3	4.5	4.9
Threonine	3.7	2.8	2.8	2.8	3.0	2.9	2.8	2.7
Valine	5.4	4.3	4.5	4.6	4.5	4.2	4.4	4.7
Nonessential								
Alanine	2.5	3.7	3.9	3.8	4.3	3.9	3.8	3.8
Aspartic acid	6.0	6.7	6.9	6.5	6.7	7.0	7.0	6.8
Cystine	0.4	1.3	1.7	1.5	0.9	1.4	1.4	1.4
Glutamic acid	16.4	16.8	18.8	17.5	14.7	16.4	18.3	18.7
Glycine	1.6	3.8	3.9	3.6	4.3	4.1	4.0	3.7
Proline	9.2	3.3	3.8	4.2	4.0	3.9	4.3	4.5
Serine	4.8	3.9	4.2	3.8	4.0	4.2	4.2	3.8
Tyrosine	5.2	3.2	3.6	3.6	3.1	3.3	3.5	3.4
NH_3	9.0	13.9	15.2	12.3	14.0	14.4	15.1	14.1
Crude protein (N × 6.25)[d]	91.5	22.8	64.8	77.6	14.9	15.5	56.2	68.0

[a] Protein extract
[b] Protein concentrate
[c] Bran fraction
[d] Crude protein content of the protein sources as is basis

and 15.5% respectively (Table 2). Eppendorfer (1977) reported that oats (var. Selma) with high nitrogen content had a high true protein digestibility; this phenomenon is typical of cereals (Youngs et al., 1982).

Scott groats had a higher PER value than Hinoat groats in spite of a lower ($p < 0.05$) protein ADC. Hence when we calculated the liveweight gain:absorbed nitrogen ratio, rats fed Scott groats had a ratio of 2.91 as compared to 2.59 for those fed Hinoat groats. This was indicative that rats fed Scott groats utilized more efficiently the absorbed nitrogen than those fed Hinoat groats. Since there was no major difference in essential amino acid pattern between the two groats (Table 2) the difference observed in PER values may be due to differences in the bio-availability of some essential amino acids.

Crude protein extract (PE). PER of Scott PE was higher ($p < 0.05$) than that of Hinoat PE and the values were respectively 72% and 67% of casein. NPR was not significantly ($p > 0.05$) different between the two PE. However, for both cultivars, PERs of PEs were lower than those of groats but no major difference in essential amino acid profile was noted (Table 2). Ma (1983a) indicated that protein may undergo some denaturation when oat isolates are prepared at pH 9.5 which might explain the lower PERs of PEs.

The ADC of Hinoat PE was higher ($p < 0.05$) than ADC of Scott PE. Within the cultivar, ADC of PE was higher ($p < 0.05$) than that of groats. Therefore solubilization of proteins at pH 9.5 improved protein ADC.

Protein concentrate (PC). PER of Scott PC was higher ($p < 0.05$) than that of Hinoat PC and the values were respectively 73% and 68% of that of casein. NPR was not significantly different between the two PC. However, for both cultivars, PERs of PCs were lower ($p < 0.05$) than PERs of groats, but, here again, no major difference in essential amino acid profile was noted (Table 2). As with PE, the loss of nutritive value as compared to groats may be due to some denaturation of proteins at pH 9.5

The ADC of Hinoat was higher ($p > 0.05$) than that of Scott PC. Similar findings were observed with groats and PEs. Within the cultivar, protein ADCs of PCs were higher than those of groats and PEs. It is apparent that isoelectric precipitation of proteins following solubilization further increased protein ADC.

Bran fraction (BF). The nutritive value of Hinoat bran, as measured by NPR, was not significantly different ($p > 0.05$) from that of Hinoat groats or Hinoat PC in spite of a lower ($p < 0.05$) protein ADC and a lower ($p < 0.05$) feed intake. This may be explained by the lysine content which was higher

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A Research Note

Proteolytic Enzymes from *Cnidoscolus chayamansa* "Chaya"

FCA. AIDA ITURBE -CHIÑAS and AGUSTÍN LÓPEZ-MUNGUIA CANALES

ABSTRACT

Proteolytic activity from "chaya" leaves was best extracted at pH 8 (0.05M phosphate buffer). Good storage stability was shown at pH 10 retaining 70% of initial activity after 30 days. Proteolytic enzymes were partially purified by isoelectric and ammonium sulfate precipitation. Optimal proteolytic activity was found at pH 7.5. Proteases were tested for their ability to hydrolyze several food proteins. At pH 7.5 the hydrolysis of urea-denatured hemoglobin was extensive; casein and soybean proteins were hydrolyzed to a moderate degree and bovine serum albumin, gelatin and fish protein isolate to a lesser degree rate.

INTRODUCTION

PROTEASES have been used traditionally in food processing. Their uses in cheese making and meat tenderizing have been extended to baking (Velazco, 1974), beer stabilization and chillproofing (Kennedy and Pike, 1981), protein beverage production (Sejr-Olsen et al., 1978, Adler-Nissen, 1978), protein isolation, etc. Vegetable proteolytic enzymes still play an important role in the food industry. Such is the case of papain, ficin, and bromelin, which are commonly used in brewing and meat tenderization.

A wild shrub named "chaya" (*Cnidoscolus chayamansa*) grows in the Southeast of Mexico, in the states of Yucatán, Tabasco and Quintana Roo, which has been traditionally considered a good source of protein (Cravioto et al., 1952, Martín et al., 1977). It has been shown that water extracts from the leaves of this plant have milk clotting activity (Mendizabal Oriza, 1980).

Considering possible commercial importance of the "chaya" proteases, the purpose of this study was to investigate the enzyme extraction and hydrolysis of some food proteins.

MATERIALS & METHODS

Enzyme extraction and purification

The selected "chaya" leaves from Villa Hermosa, Tab. (Méx.) were macerated with different buffer solutions (0.05M phosphate, pH 6, 7 and 8; 0.05M tris-HCl pH 8 and 9; 0.05M carbonate pH 9, 10 and 11) in a ratio 1:7.5 and stirred for 1 hr at 4°C. After coarse filtration, 1% polyvinylpyrrolidine (PVP) was added to the filtered solution and was then centrifuged at 14,000 × *g* for 15 min. The supernatant was adjusted to pH 10, as previous experiments showed a 3.75-fold increase in specific proteolytic activity with this isoelectric precipitation treatment, probably due to precipitation of enzyme inhibitors. The inactive precipitate was removed by centrifugation (10,000 × *g*, 15 min). The proteolytic activity was precipitated from pH 10 solution with solid ammonium sulfate at 55% saturation at 4°C in one step. The precipitated protein, recovered by centrifugation at 15,000 × *g* for 30 min, was dissolved in 0.05M carbonate buffer pH 10 and dialyzed against the same solution. The final solution containing 9.35 mg protein/mL was frozen and used as the partially purified "chaya" proteases (PCP) in all experiments.

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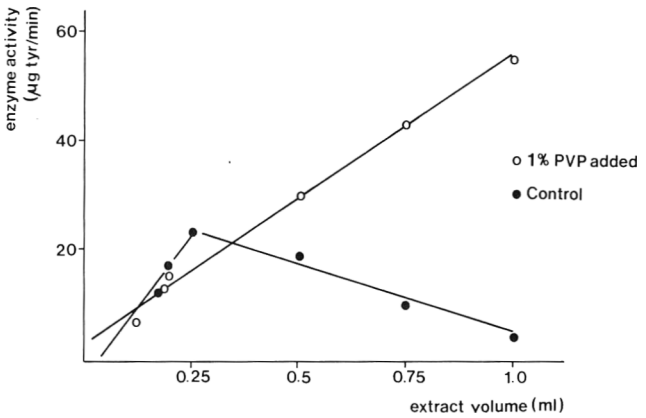

Fig. 1 — Stabilization effect of polyvinyl pyrrolidine (PVP) on enzyme activity at different enzyme concentration (40°C, pH 7.5).

Optimum pH

To determine the optimum pH of the PCP activity, the substrate [2% urea-denatured hemoglobin (Bioxón)] was prepared in different buffer solutions (0.05M phosphate pH 6, 7 and 8; 0.05M Tris-HCl pH 8 and 9; 0.05M carbonate pH 9, 10 and 11) to obtain a pH range between 6 and 11.

Protease assay

Proteolytic activity was determined by Anson's method (Anson, 1938) measuring reactive amino acids with the Folin-Ciocalteau reagent after TCA precipitation, with 2% urea-denaturated hemoglobin as standard substrate. At pH 7.5 and 40°C, 1 mg PCP gave 2.318 μg tyr/min.

A similar procedure was followed to measure activity of PCP with other proteins: bovine serum albumin (BSA, Sigma Chem. Co.), Hammerstein casein (ICN Pharmaceuticals), food grade casein (Arancia, S.A. Méx.), commercial soybean flour, gelatin and fish protein isolate, using a 1% protein concentration. Protein was determined by the Lowry method (Lowry et al., 1951) using bovine serum albumin as standard.

To measure degree of hydrolysis (DH) of hemoglobin, an 8% water solution was reacted in a pH-stat. Initial pH was adjusted to 7.5 and reaction was started with the addition of the enzyme solution. The pH was kept constant by contiunous addition of 0.5N sodium hydroxide. The DH was calculated using Adler-Nissen's equation (1977), with a pK of 7.1 and considering a total number of peptide bonds for hemoglobin of 8.38 eq/kg.

RESULTS & DISCUSSION

WITT AND TOUSIGNAT (1967) reported extraction of malt proteases at pH 6 in 5% K_2SO_4. The "chaya" proteases were extracted with buffers in the range pH 6–11, since initial experiments at pH 6 gave low values of enzyme activity compared with the activity of the same solutions at pH 10. Optimum extraction was found at pH 8.0 with phosphate buffer.

The stabilization effect of PVP on the proteolytic activity of the "chaya" leaves solution is shown in Fig. 1. At higher enzyme concentrations there was a decrease in enzyme activity, probably due to the effect of polyphenolic compounds. These compounds, mainly the tannin group, were reported to

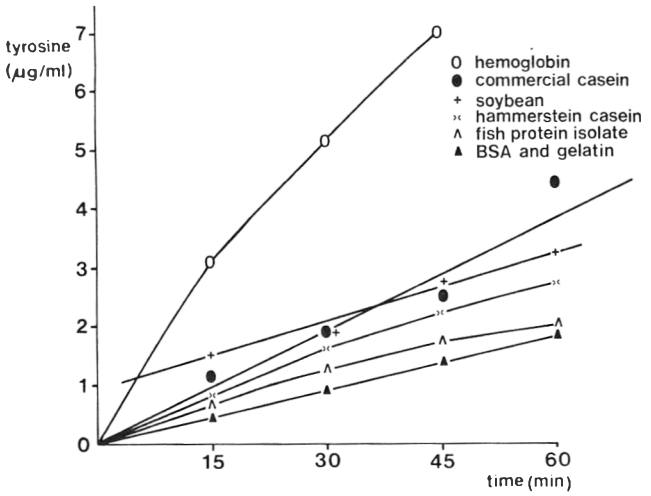

Fig. 2 — Activity of "chaya" proteases (PCP) for some common food proteins (40°c, pH 7.5, 1% substrate, concentration and 0.52 mg/mL of PCP).

act as enzyme inhibitors, inactivating a great variety of enzymes (Feeney, 1969). The addition of PVP had a stabilizing effect by removing phenolic impurities. When PVP was added the reaction rate was linear with enzyme concentration.

The optimum pH of activity was found between 7.5 and 8.5 in 0.05M Tris-HCl buffer. The proteases can be considered as neutral or slightly alkaline. The enzyme showed maximum stability at 4°C (pH 10.0) retaining 70% of the original activity after 30 days.

In Fig. 2 the activity of "chaya" proteases (PCP) on some common food proteins is shown. All substrates were compared under the same conditions (40°C, pH 7.5). The proteases were highly active toward hemoglobin and showed lower activities for commercial casein, soybean flour, Hammerstein casein, fish protein isolate, bovine serum albumin and gelatin.

The milk clotting activity was very slow compared with that of commercial rennin, probably due to the difference in pH between milk and that of optimal proteases activity. For the other substrates, the use of "chaya" proteases may be considered when a modification of functional properties is desired. For example, "chaya" protease may be employed for hydrolysis-decoloration of hemoglobin proposed by Sejr-Olsen (1980). In terms of degree of hydrolysis (25%) and characteristics of the final product (solubility and decoloration), the PCP is very well suited for this process.

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in bran than in groats or PC (Table 2). Ma (1983b) also found a higher concentration of lysine in Hinoat and Sentinel oat bran fractions compared to groats or concentrates.

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A Research Note

Effect of Oligosaccharide Formation on the Cryoscopic Measurements of Enzymatic Hydrolysis of Lactose in Dairy Products

I.J. JEON and S.R. SAUNDERS

ABSTRACT

The effect of oligosaccharide formation on the cryoscopic measurements of enzymatic hydrolysis of lactose was evaluated. Whey permeate, 5% lactose solution, and milk samples were treated with β-galactosidase and incubated for 4 hr at 37°C. Samples were withdrawn at regular intervals and analyzed for the degree of hydrolysis by a freezing point depression method (FPD), as well as by high performance liquid chromatography (HPLC). Results indicated that the percent hydrolysis of lactose was substantially less with the FPD measurement than with HPLC. Differences between the two analyses were coincided with the levels of oligosaccharides formed by transgalactosidation reactions during hydrolysis.

INTRODUCTION

USE OF THE FREEZING POINT measurement to determine the degree of enzymatic hydrolysis of lactose in dairy products has been suggested by a number of researchers in recent years. These products include milk (Nijpels et al., 1980; Chen et al., 1981), whey (Baer et al., 1980), whey permeate (Nijpels et al., 1980; Chen et al., 1981), and ice cream mix (Frank and Christen, 1984). All of these analyses were based on the assumption that hydrolysis of lactose by β-galactosidase to its monosaccharide components, glucose and galactose, would yield a depression of the freezing point of the solution in direct proportion to the moles of monosaccharides released (Nijpels et al., 1980). To make this assumption work, all lactose must be hydrolyzed to its monosaccharides with insignificant formation of oligosaccharides by transgalactosidation. However, it is well known that a number of oligosaccharides are formed by galactosyl transfer reactions during the hydrolysis of lactose by β-galactosidase (Richmond et al., 1981). According to a recent study with high performance liquid chromatography (HPLC), a considerable amount of oligosaccharides is produced as a result of the transgalactosidation reactions when the enzyme acted on lactose (Jeon and Mantha, 1985). This study was undertaken to evaluate the significance of oligosaccharide formation on the measurement of hydrolysis of lactose by freezing point depression (FPD).

MATERIALS & METHODS

SAMPLES of 5% lactose solution in 0.025M potassium phosphate buffer (pH 6.60), whey permeate from a local dairy plant, and whole milk from the Kansas State University dairy plant were inoculated with a commercial β-galactosidase from *Candida pseudotropicalis* at 1 μmole orthonitrophenol (ONP) unit per mL. They were incubated at 37°C for 0, 0.5, 1.0, 2.0, 3.0, and 4.0 hr and then immediately heated in an 80°C water bath for 10 min to stop enzyme actions. The depression of freezing points of each sample was measured using a Fiske Milk Cryoscope Model J-61 (Jeon and Bassette, 1982). The freezing point standards were 7.0 and 10.0% sucrose solutions, which were prepared and used according to AOAC (1980) procedures. The cryoscopic readings (Hortvet, H) were converted to Celsius (°C) scale by the equation, $C = -0.0011558 + 0.964824H$ (Demott, 1982). The percent hydrolysis of lactose in the sample was computed assuming that the complete hydrolysis of a 5% lactose solution would theoretically result in a FPD of −0.273°C (Nijpels et al., 1980). Triplicate measurements were made for each sample.

A Beckman 100A system with an Altex Model 156 refractive index detector was used for all HPLC analyses. Glucose and galactose as well as lactose and other oligosaccharides were separated on an Amino Spheri-5 column (Brownlee Labs, Santa Clara, CA), as described by Jeon and Mantha (1985). Whey permeate samples were prepared for HPLC analysis by the procedure of Jeon et al. (1984), and milk samples by the method of Kwak and Jeon (1985). The 5% lactose solutions were injected without any preparations. Percent hydrolysis of lactose was calculated by determining the initial amount of lactose in the sample and that remaining at each period of hydrolysis. All HPLC analyses were in duplicate.

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RESULTS & DISCUSSION

FIGURE 1 ILLUSTRATES the formation of oligosaccharides by a transgalactosidation reaction during hydrolysis of lactose by β-galactosidase. As reported previously (Jeon and Mantha, 1985), at least five oligosaccharides were formed by the transgalactosidation, including four disaccharides (peaks 7, 9, 10, and 11) and a trisaccharide (peak 12). These oligosaccharides were reported to be galactopyranosyl D-glucose, galactopyranosyl D-galactose, and galacto-galactopyranosyl D-galactose, possibly having 2-O-β, 3-O-β, or 6-O-β linkages. Among the five oligosaccharides, only two (peaks 10 & 12) showed a substantial increase in concentration during hydrolysis. Since these oligosaccharides would not contribute to the depression of freezing points during lactose hydrolysis, their presence would cause less freezing point depression than a simple breakdown of lactose to glucose and galactose with no transgalactosidation products formed. Obviously, the adverse effect of these oligosaccharides on the depression of freezing points will depend upon the amount of the oligosaccharides formed. Table 1 shows a comparison of percent hydrolysis of lactose in 5% lactose solution, whey permeate, and milk, as measured by HPLC and FPD. The percent hydrolysis measured by FPD was substantially less than that measured by HPLC (except for 5% lactose at 1.0 hr). During hydrolysis, differences between the two measurements ranged from 2.9–6.7% for 5% lactose solution, 5.5–11.8% for whey permeate (FPD reading at 0.5 hr excluded), and 4.8–13.3% for milk samples. HPLC analysis indicated that the differences were correspondingly larger with the formation of increasingly higher concentrations of oligosaccharides. According to the oligosaccharide concentrations expressed as % lactose by refractive index, approximately 4.3–11.5% of the total lactose hydrolyzed was utilized for the formation of oligosaccharides. These amounts of oligosaccharides appeared to cause the differences between the HPLC and FPD measurements. Therefore, to make FPD measurements more acceptable for the estimation of lactose hydrolysis, it appears that the level of oligosaccharide formation during hydrolysis should be considered seriously. We found in previous studies that the level of oligosaccharide formation was influenced considerably by many factors, such as source and amount of enzyme, substrate concentration, and time and temperature of hydrolysis (Jeon and Mantha, 1985; Kwak and Jeon, 1985).

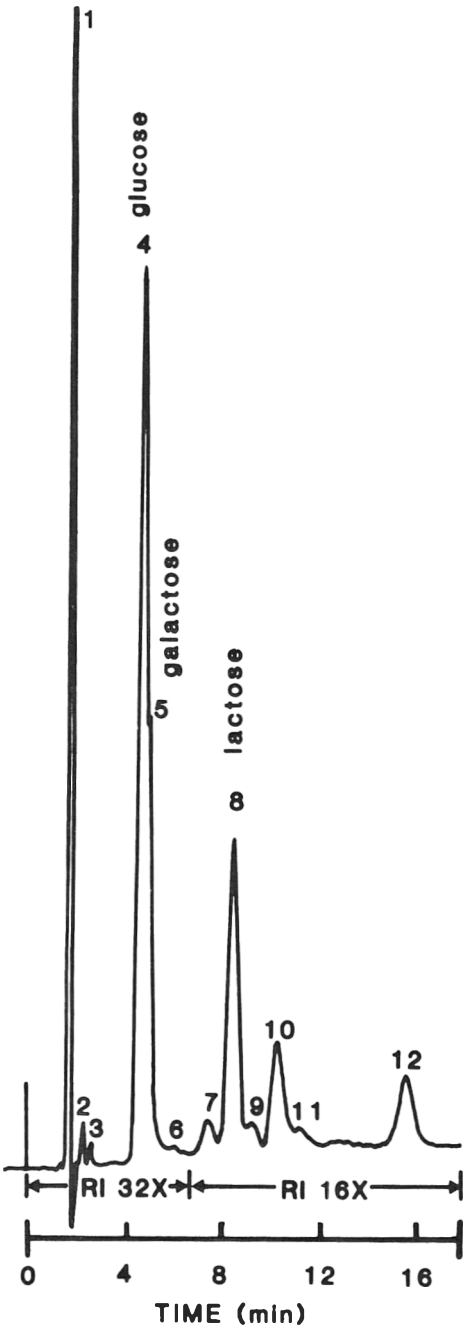

Fig. 1—High performance liquid chromatography chromatogram of whey permeate after incubating 4 hr at 37°C with 1 μmole orthonitrophenol unit β-galactosidase. Amino Spheri-5 column; solvent, acetonitrile/water (75/25); flow rate, 2.0 mL/min; RI detector. Peaks 7, 9, 10, and 11, disaccharides, and peak 12, trisaccharide; all formed by transgalactosidation.

Table 1—Comparison of percent hydrolysis of lactose in various products as measured by high performance liquid chromatography (HPLC) and freezing point depression (FPD) methods

Dairy product	Time of hydrolysis (hr)[a]				
	0.5	1.0	2.0	3.0	4.0
	%[b]				
5% lactose:					
HPLC	30.0	42.3	61.5	72.3	79.2
FPD	27.1	42.5	56.4	68.1	72.5
Difference[c]	2.9	−0.2	5.1	4.2	6.7
Whey permeate:					
HPLC	15.2	30.8	51.0	66.8	74.8
FPD	2.2	25.3	40.6	55.0	64.1
Difference	13.7	5.5	10.4	11.8	10.7
Whole milk:					
HPLC	26.8	37.5	64.3	80.0	88.1
FPD	22.0	31.9	57.5	66.7	75.5
Difference	4.8	5.6	6.8	13.3	12.6

[a] Hydrolyzed with 1 μmol orthonitrophenol unit enzyme/mL sample at 37°C.
[b] Each data point indicates a mean of duplicate for HPLC and triplicate for FPD.
[c] Subtracted FPD data from HPLC.

Therefore, we suggest that these factors should be controlled consistently for each analysis, in addition to compensating FPD for the amount of oligosaccharides formed.

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A Research Note

Hot Pork Processing, Brine Chilling and Mechanical Portioning as a Fresh Pork Processing System

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ABSTRACT

Rapid chilling was investigated as a link between hot pork processing and mechanical portioning. Loins from market weight hogs (n = 40) were removed (pre- or post-rigor) and were assigned to one of two chilling treatments (brine chilling or blast freezing). Loins were chilled until crust frozen, then tempered, pressed and cleaved. Ultimate pH, cooking yields, taste panel ratings and Warner-Bratzler shear force values (WBS) were obtained. Pre-rigor chops had significantly higher WBS values and lower (less tender) taste panel ratings than post-rigor chops. Generally, pre-rigor chops were juicier but less tender. There were no major differences between rapid chilling treatments nor in ultimate pH or cooking data. Less severe chilling techniques are needed to make this processing system feasible.

INTRODUCTION

MANDIGO and co-workers (Mandigo and Hendrickson, 1966a,b; Mandigo et al., 1977) have demonstrated that cured meats can be produced from hot boned pork. Yields of traditional primal cuts are essentially unchanged in such a system (Mandigo et al., 1979). However, it is difficult to maintain the shape of pre-rigor meat. Hinnergardt et al. (1973) used stainless steel molds in which hot boned pork loins were frozen to maintain their shape. Alternative solutions to the problem of shape are needed.

Mechanical portioning (pressing and cleaving) allows for rapid processing of pork loins and does not alter tenderness (Savell et al., 1980; Goldner and Mandigo, 1974). Portioning could be added to an accelerated processing system if the shape of the boneless loin could be maintained. Crenwelge et al. (1984) used brine chilling to rapidly chill pork carcasses. Using such a technique to crust freeze pork loins should maintain loin shape. The objective of this research was to assess the feasibility of brine chilling as a means of linking accelerated pork processing to mechanical portioning.

MATERIALS & METHODS

BONELESS LOINS (longissimus muscle) were removed from conventionally slaughtered, market weight hogs (n = 40). Left loins were removed pre-rigor (1 hr post-stunning) and right loins were removed post-rigor (24 hr post-stunning) after cooling in a 2°C cooler. The loins were protectively covered before crust freezing (firm on the surface, soft in the center) by one of two chilling methods: brine chilling with an 88 salometer brine at −23°C or blast freezing at −30°C. Loins to be brine chilled were vacuum packaged in oxygen and moisture barrier bags and loins for blast freezing were wrapped in polyvinylchloride film. Chilling times were about 2 hr for brine chilling and 3 hr for blast freezing. Since it was necessary to vacuum package brine chilled loins, chilling time and subjective observations, instead of temperature, were used to determine when muscles were crust frozen.

Pairs of pre- and post-rigor loins were randomly assigned to four groups (10 pairs each) to allow comparisons of chilling methods across rigor states. The four groups were: brine chilled, pre-rigor versus post-rigor; pre-rigor, brine chilled versus post-rigor, blast frozen; pre-rigor, blast frozen versus post-rigor, brine chilled; and blast frozen, pre-rigor versus post-rigor. After crust freezing, the loins were tempered in a −2°C cooler for 24 hr and 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 chops with a Model 81 Bettcher Power Cleaver (Bettcher Ind., Inc., Vermilion, OH). Chops were vaccum packaged in oxygen and moisture barrier bags and frozen at −30°C for later analyses.

All chops were weighed frozen, then thawed for 24 hr in a 0–2°C cooler. Thawed chops were removed from the vacuum package, blotted lightly on paper towels and weighed to determine thaw loss. The chops were oven roasted at 177°C to an internal temperature of 70°C (turned over at 35°C), measured with copper-constantan thermocouples inserted into the center of the chops. Cook yields were determined by weighing the chops before and immediately after roasting.

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 cooling to room temperature (about 1 hr), the chops were trimmed and four to eight 12.5 mm diameter cores (following the fiber grain of the chop) were taken. A 500 kg load cell and a 50 kg load range were used with a crosshead speed of 250 mm/min to shear each core. No core was sheared more than twice. Results are expressed as kg of force required to shear sample cores.

An eight member, trained (Cross et al., 1978), sensory panel evaluated the parameters of tenderness and juiciness on a 15 cm unstructured line scale. Panelists rated samples by placing a horizontal line 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. The panel was presented six randomly selected samples per session under red lights. Each loin was evaluated one time by the panel. Temperature of the samples was maintained using the 50°C double boiler technique of Olson et al. (1980).

The data were analyzed by the paired-t distribution to determine the significance of differences between treatments within groups. Statistical analyses were obtained from the statistical analysis system of Barr et al. (1979).

RESULTS & DISCUSSION

TABLE 1 presents shear forces, taste panel ratings, thawing losses and cooking losses of chops from pre- and post-rigor, paired pork loins that were brine chilled or blast frozen. In all Warner-Bratzler shear force comparisons and in three of the four comparisons of taste panel tenderness ratings, chops from pre-rigor, rapidly chilled loins were less tender than chops from post-rigor, rapidly chilled loins. These results are similar to those found in other studies involving pre-rigor processed pork (Weiner et al., 1966; Hinnergardt et al., 1973). Either cold shortening or thaw rigor occurred and the resulting muscle shortening may have caused the reduction in tenderness (Galloway and Goll, 1967; Davey and Gilbert, 1974; Follett et al., 1974).

Thaw and cooking losses for the post-rigor chops were equal or greater than the pre-rigor chops (Table 1). Since thaw rigor is generally associated with an increase in drip (thaw) loss and since the post-rigor chops could not have undergone thaw rigor because of a 24 hr chill prior to crust freezing and since no

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Table 1—Traits of chops from pre- or post-rigor, paired pork loins that were brine chilled or blast frozen

Trait	Pre-rigor			Post-rigor	
	Brine chilled	Blast frozen		Brine chilled	Blast frozen
WBS[a]	4.67		**	3.34	
	(0.70)			(0.84)	
	5.38		*		4.41
	(0.95)				(0.92)
		5.40	*	4.03	
		(1.68)		(0.94)	
		5.26	*		3.71
		(1.91)			(1.09)
Tenderness[b] ratings	7.94		**	10.20	
	(2.55)			(2.90)	
	7.38		**		9.17
	(2.60)				(3.01)
		9.05		9.23	
		(2.28)		(2.50)	
		7.69	**		10.17
		(3.97)			(3.01)
Juiciness[c] ratings	6.62			7.35	
	(2.90)			(2.28)	
	9.28		*		8.01
	(2.44)				(2.30)
		9.06	*	7.57	
		(2.75)		(2.38)	
		8.81	**		6.96
		(1.06)			(2.10)
Thaw loss %	8.42			7.86	
	(2.91)			(3.60)	
	5.83				7.20
	(3.70)				(3.52)
		5.71	*	8.54	
		(3.11)		(1.83)	
		6.19			7.87
		(2.82)			(3.03)
Cook loss %	19.86			22.23	
	(3.72)			(5.43)	
	20.89				21.55
	(4.32)				(3.12)
		21.02		21.06	
		(4.98)		(3.90)	
		20.20			19.05
		(1.74)			(4.29)

[a] Warner-Bratzler shear force expressed as kg force required to shear a 12.5 mm diameter sample core (higher values are less tender).

[b] Tenderness ratings based on a 15 cm unstructured line scale (0 = tough, 15 = tender).

[c] Juiciness ratings based on a 15 cm unstructured line scale (0 = dry, 15 = juicy).

* Means in the same row are significantly different ($P<0.05$).

** Means in the same row are significantly different ($P<0.01$).

Parenthetical values are standard deviations.

differences were observed for thaw loss of pre- and post-rigor chops, then cold shortening was probably cause for the tenderness differences. Although Bendall (1975) suggested that cold shortening is not much of a problem in pork, recent research has shown that pork can cold shorten when the conditions are sufficiently severe (Dransfield and Lockyer, 1985).

In three of the four sensory comparisons (Table 1), pre-rigor processed chops were juicier than post-rigor processed chops. These results agree with data collected from pre-rigor processed pork (Mandigo and Hendrickson, 1966a). However, other studies with pre-rigor processed pork and beef showed no beneficial results to juiciness (Schmidt and Gilbert, 1970; Marriott et al., 1980).

Research with beef and lamb indicates that delaying the chilling of pre-rigor muscle results in acceptable tenderness (McCrae et al., 1971; Kastner and Russell, 1975). Perhaps the detrimental effect on tenderness of crust freezing pre-rigor pork loins could be overcome by lengthening the time from slaughter to initiation of the rapid chilling technique. Such an approach would allow for the depletion of more of the ATP within the muscle and thus would lessen the risk and/or severity of cold shortening.

In conclusion, brine chilling, when applied within 1 hr post-stunning, is detrimental to tenderness of pork. Research is needed on use of less severe chilling techniques or a longer delay between stunning and rapid chilling to overcome the tenderness problem and to allow for linkage of rapid chilling and mechanical portioning.

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A Research Note

A Suggested Instrumental Technique for Studying Dynamic Flavor Release from Food Products

WILLIAM E. LEE III

ABSTRACT

An instrumental technique is outlined which can be used to study the dynamic volatile flavor release from various food systems. A mass spectrometer coupled with a specially designed apparatus is utilized. The apparatus provides the capability to study several aspects of the in-mouth environment including thermal, work input, and salivation effects. Monitoring of the headspace concentration is essentially continuous. The technique yields accurate reproducible results and should be applicable to a wide range of food systems.

INTRODUCTION

THE DYNAMIC RELEASE of flavor as it occurs in the mouth environment is a very important but poorly understood area. Investigation is difficult due to the complicated phenomena which occur over very short time spans. Sensory techniques have been developed to measure time-related responses (Larson-powers and Pangborn, 1978; Pangborn and Koyasako, 1981). Such techniques must have a sufficient number of panelists to insure accuracy. In general, factors such as age, varying saliva composition and flow, the onset of fatigue, and other parameters vary from judge to judge and can complicate the data analysis. Also, sensory techniques require that the samples be palatable. The purpose of this work was to develop an instrumental technique to study dynamic volatile release which would greatly reduce the potential difficulties associated with the sensory methods.

MATERIALS & METHODS

Experimental apparatus

The method uses a conventional GC/MS system. An experimental apparatus as shown in Fig. 1 basically replaces the GC column. The apparatus incorporates several features. First, the temperature of the sample environment can be accurately controlled by the appropriate use of water baths. This allows the mimicking of heat transfer to or from the foodstuff which occurs within the mouth environment. Second, solutions such as artificial saliva can be injected as desired through the vial septum cap. This permits the modelling of salivation. Third, small metal or glass balls can be placed inside the vial along with the sample. When agitated side-to-side, this mimics the work input effects of mastication. Finally, the vial is continuously flushed with inert gas, typically helium. Sending this gas to the mass analyzer allows the monitoring of the vial headspace composition as a function of time.

The vial was a 1.5 mL glass vial (American Scientific Products C4800-6) with a wall thickness of approximately 1 mm. The helium flow rate was 50 mL/min which gave a flow pattern very close to plug flow. At 50 mL/min, the vial is being flushed approximately 33 times per min. Standard 8 mm Teflon-faced silicone rubber septa were utilized.

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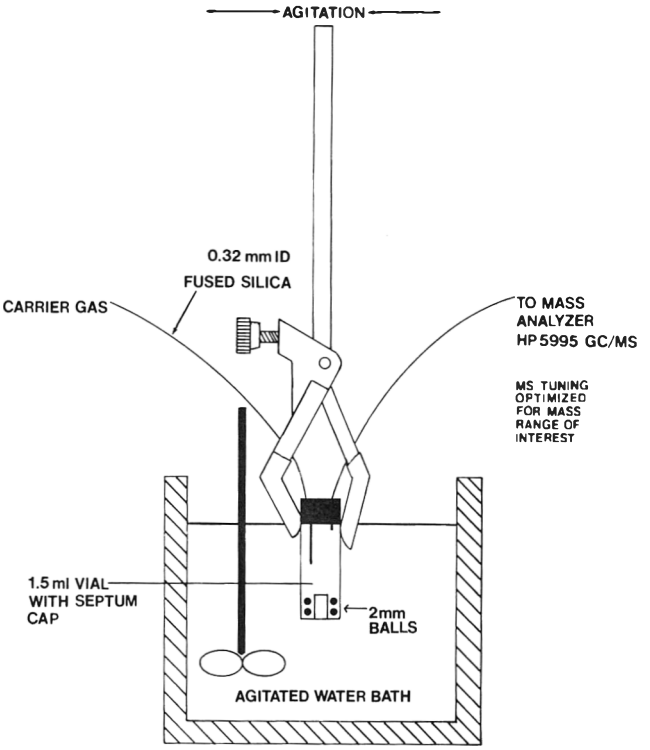

Fig. 1—Diagram of experimental apparatus utilized in the instrumental technique. The sample is located within the 1.5 mL vial. The apparatus replaces the GC column within the GC/MS instrument.

Temperature control

Two temperature baths were utilized. The first bath was held at 0 ± 0.2°C. and the second at 35 ± 0.2°C. The two baths were used to allow the modelling of the situation where a cool foodstuff is placed into the warmer mouth environment.

Work input

Four metal balls of 2 mm diameter were used. A side-to-side agitation rate of approximately four shakes per second was used with the agitation path being 8 cm. Higher agitation rates were not possible; the upper agitation limit appears to be set by the ability of the vial septum to maintain a seal around the inlet and outlet carrier gas tubes.

Mass spectrometer

Experiments were carried out on a Hewlett Packard model 5995 quadrupole mass spectrometer equipped with an HP microprocessor. A mass range is selected (41 to 200 in the present work) so that the ion current of all molecular fragments within the range is measured. This can be thought of as measuring "total volatiles." The data are stored on discs for subsequent analysis by the HP software.

Typically, the electron multiplier is set at 1800–2000 volts with a scanning rate of 380 amu/sec. The system optics is tuned to emphasize the mass range of interest. It is important to set all post-vial line temperatures high enough to reduce potential condensation.

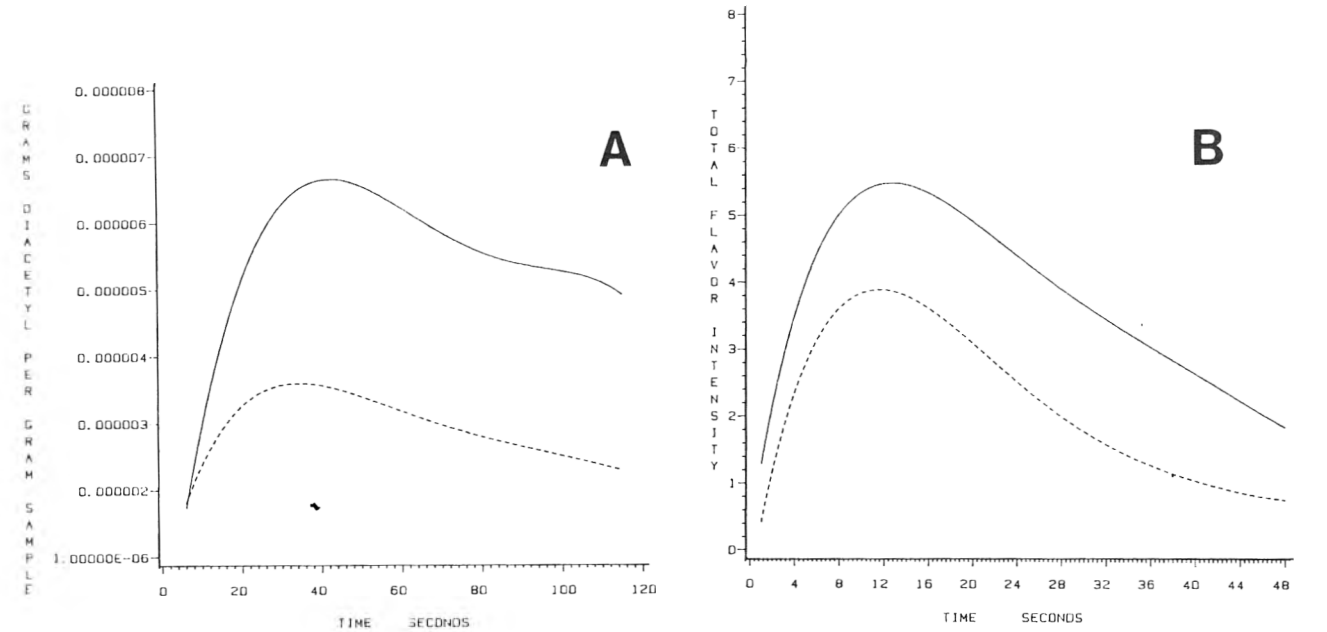

Fig. 2—Instrumental (A) and sensory (B) evaluation of flavor intensity release from the olein palm fraction (———) and stearin palm fraction (- - - -) samples. For the sensory evaluation, 0 = "none," 5 = moderate," and 10 = "a very great deal."

Experimental procedure

Following the sample preparation, the sample (0.10 ± 0.02g) is allowed to equilibrate at the starting temperature of 0°C. An MS output base line is established during this time. At time t_0: (1) 0.25 mL artificial saliva (at 35°C) is injected; (2) the vial is immersed in the 35°C bath; and (3) vial agitation is initiated. Data acquisition typically lasts for 2–3 minutes after t_0.

Artificial saliva

The artificial saliva consisted of 0.40% gastric mucin, 0.42% K_2HPO_4, 0.32% KH_2PO_4, 0.03% KOH, 0.02% KCl, 0.02% NaCl, 0.001% Na_2SO_4, 0.001% $MgCl_2$, 0.006% urea, 0.04% amylase, 0.37% dried egg white, and 98.372% distilled deionized water.

Sensory time-intensity measurements

The sensory technique used to measure total flavor intensity is described elsewhere (Neilson, 1957). Eight trained judges participated. The sample size was 2.5 ± 0.1g with the samples initially equilibrated at 0°C. At t_0, the judges placed the sample into their mouth and evaluated "total flavor" until the intensity returned to zero following swallowing. Replicate evaluations were done by all judges.

Sample preparation

The model system studied consisted of samples formulated with two kinds of palm fat. A stearin (high melting) and olein (low melting) fraction was used in order to study the influence of fat physical properties on the flavor release. Instrumental method samples were prepared using mixtures of 40% palm fat, 0.05% diacetyl and the remainder liquid soybean oil. The mixtures were completely melted then recrystallized at a storage temperature of 0°C.

Sensory evaluation samples were prepared using the same levels of fat and oil. The flavor system consisted of approximately 0.01% diacetyl and other dairy-like flavors. This was done to make the samples more palatable.

RESULTS & DISCUSSION

Figure 2A presents the results of the instrumental evaluation. The olein curve is the average of 7 runs and the stearin curve is the average of 6 runs. The individual curves were not statistically different from the average curves ($\alpha = 0.10$). The olein curves are statistically different from the stearin curves ($\alpha = 0.01$). The results demonstrate the expected result: the high solids content/slow melting rate stearin sample releases less flavor than the low solids content/fast melting rate olein sample.

The sensory evaluation presented in Fig. 2B demonstrates the same trend. The individual response curves were averaged to a single response curve for each sample. The individual curves were not statistically different from the averaged curve ($\alpha = 0.10$) but the olein curve was statistically different from the stearin curve ($\alpha = 0.01$). The sensory and instrumental results cannot be compared rigorously since the flavor system is not exactly identical (however, the structural fat is identical in both cases). Also, parameters such as the ratio of saliva to sample at any point in time are not equivalent. However, the instrumental method gave results that at least qualitatively followed the trends observed in the sensory evaluation.

Obviously, more work would have to be done to establish the utility of the instrumental technique. Other food systems representing a range of properties and incorporating a spectrum of volatile flavor classes would need to be investigated.

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A Research Note

Implication of Breeding Programs on Potato Quality

BARRY GOSSELIN and NELL I. MONDY

ABSTRACT

The effect of potato breeding on the tendency of tubers to bruise or discolor as well as the phenolic content of tubers was examined. A new cultivar, Lemhi Russet, was compared with two well established varieties, Katahdin and Russet Burbank. In addition, two new nematode-resistant cultivars, Hampton and Islander, were compared with a nematode-susceptible variety, Katahdin. Lemhi was found to discolor more and was higher in phenols than either Katahdin or Russet Burbank. The nematode-resistant varieties, Hampton and Islander, discolored less and were lower in phenols than Katahdin, a nematode-susceptible variety.

INTRODUCTION

POTATOES are a major component of the U.S. diet. In 1983, the annual per capita consumption was 121.2 lb (USDA, 1984). However, potato tubers are subjected to considerable losses due to disease, pests and physiological defects. Plant breeders have the task of developing new cultivars which are resistant to disease, pests, and physiological disorders and result in high yields. In addition to these objectives, breeders should also consider tuber quality. Quality factors to be considered in evaluating any food are color, flavor, texture, nutritive value, and freedom from toxic compounds. It is important that each of these factors be considered by plant breeders when developing new cultivars for market.

Enzymatic discoloration of raw potatoes occurs when potatoes are cut or bruised and this makes the potato unappealing to consumers. It is one of the most serious and costly problems in the potato industry. This type of discoloration has been shown to be positively correlated ($r = +0.97$) with tuber phenolic content (Mondy et al., 1979). In addition, phenolic content has also been shown to be positively correlated with off-flavors such as bitterness and astringency (Mondy et al., 1971) as well as after-cooking blackening (Mulder, 1949; Thomas, 1981).

The purpose of this investigation was to compare the quality of new cultivars with the well-established varieties for tendency to discolor and for phenolic content.

MATERIALS & METHODS

THE CULTIVARS used in this study were Lemhi Russet (developed in Idaho and released September, 1980), Hampton (developed in NY and not yet released), Islander (developed in Maine and Long Island and released in February, 1983), and two well known cultivars, Russet Burbank and Katahdin. Potatoes were grown at the Cornell Vegetable Research Farm in Freeville, NY and were stored in the dark at 5°C until sampled. In both years of the study tubers were analyzed shortly after harvest, and in the second year tubers were also analyzed following 4 months of storage. In 1983, the new cultivar, Lemhi Russet, was compared with the Katahdin and Russet Burbank, two well known cultivars. Two nematode-resistant varieties, Hampton and Islander, were compared with the susceptible variety, Katahdin.

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Four potatoes were randomly selected for each determination and duplications were made on each cultivar at each storage period. Potatoes were cut longitudinally from bud to stem to include both apical and basal ends. Subsequent slices were separated into cortex and pith sections.

Determination of phenols

Phenolic content was determined colorimetrically on fresh cortex tissue, including the periderm, as described by Mondy et al. (1967). Four determinations were made on each cultivar.

Determination of color

Discoloration was determined using the Hunter Color Difference Meter as described by Mondy et al. (1967). Three determinations were made on each cultivar.

RESULTS & DISCUSSION

LEMHI RUSSET was found to discolor more and was higher in phenolic content than either Katahdin or Russet Burbank (Fig. 1). Pavek et al. (1981) also reported that Lemhi was susceptible to bruising and blackspot. Both russet cultivars, Lemhi and Russet Burbank, discolored more and were higher in phenolic content than the round white potato cultivar, Katahdin. The higher tendency of Lemhi to discolor has caused problems for both growers and processors. This tendency is probably due to its higher phenolic content since Mondy et al. (1979) found a high positive correlation ($+0.97$) between discoloration and phenolic content. In addition, a high phenolic content has been shown to cause bitter and astringent off-flavors in potatoes (Mondy et al., 1971).

Two nematode-resistant varieties, Hampton and Islander, were found to discolor less and were lower in phenolic content than the nematode-susceptible cultivar, Katahdin (Fig. 2). This was true immediately following harvest and after 4 months of storage. These findings are in agreement with those of Mondy et al. (1985) who studied several nematode-resistant cultivars. Therefore, breeding for nematode resistance not only increased resistance of the potato plant itself but also resulted in im-

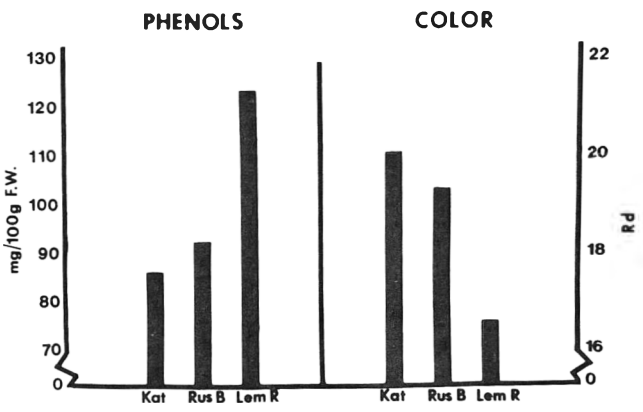

Fig. 1—Phenolic content and color of Katahdin (Kat), Russet Burbank (Rus B) and Lemhi Russet (Lem. R) cultivars. Reflectance (Rd) values are inversely related to discoloration.

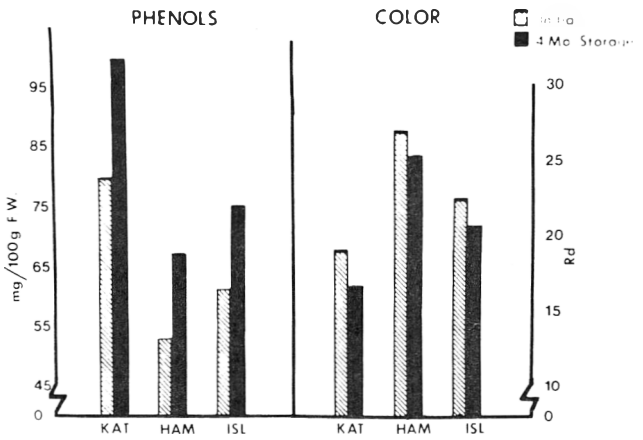

Fig. 2—Phenolic content and color of Katahdin (Kat), Hampton (Ham) and Islander (Isl) cultivars. Reflectance (Rd) values are inversely related to discoloration.

proved potato quality by decreasing the susceptibility to discoloration and reducing phenolic content.

The phenolic composition of the tuber is just one example of how the chemical composition of the tuber affects its quality. Since breeding alters the chemical composition of the tuber, it is very important that potato breeders give careful consideration to tuber quality in all breeding programs.

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Erratum Notice

J. Food Science 50(5): 1508–1509. Extrusion of Wet Corn Gluten Meal by M. Bhattacharya and M.A. Hanna. On page 1508, Introduction, second paragraph, line 9, 0.03 should have read 0.3. Please change accordingly.

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