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The Society for Food Science and Technology

Research Notes

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Volume 56: Number 6

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Memo *FROM THE SCIENTIFIC EDITOR*

Innovations Improve Quality and Increase Efficiency

WITH INNOVATIONS initiated last year, we have continued to improve the scientific quality of manuscripts printed in the *Journal of Food Science* and the efficiency of the review/revision process. Our new system of eight Associate Scientific Editors has continued to function more smoothly and is proving to be effective in upgrading the scientific merit of accepted manuscripts.

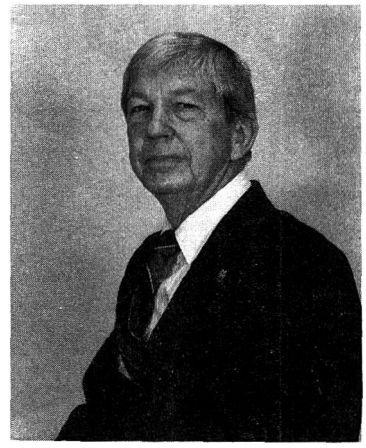
In general, manuscripts are in much more acceptable condition upon receipt than they had been in the past. Even though our rejection rate has increased from 40% to 54%, we are rejecting fewer for reasons of inappropriateness or major format problems. We believe this is, at least partly, a result of two new programs — the Editors Forum held at the IFT Annual Meeting in Dallas, and the Memos from the Associate Editors which have been published in each issue this year. If you missed the Memos, look back. You'll find some excellent pointers which will help you submit your manuscripts in the best condition, and greatly cut the time between submission and printing.

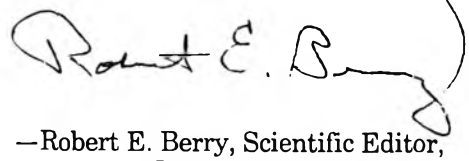
THE EDITORS FORUM on "How to Get Your Paper Published in the *Journal of Food Science*," presented some very worthwhile, educational, and practical information. Proceedings of the Forum will be available within the next months. Please obtain a copy as soon as it is published. If you keep the proceedings at your side when you are preparing your manuscript and refer to it often, you may greatly reduce the time required for your manuscript to be published.

ANOTHER CHANGE begins in 1992. After December 31, 1991, we will no longer accept Research Notes, as such, for publication. Those that are already in the review process will be published as Research Notes, but any submitted after that date will be handled as regular manuscripts, *i.e.* they will not be handled with priority.

In the place of Research Notes, we are initiating Priority Reports. These will have no length restrictions, but they must be on a very important subject and be extrememly innovative or unique. Thus, the first requirement will be that they must be *original*. Also, classification as a Priority Report must be requested by the author — with a few lines of justification — and must have the concurrence of the reviewers, Associate Editor, and Scientific Editor. Priority Reports will be given priority over other manuscripts submitted for publication and will be published within 4–6 months of submission. They will be placed in a special section of *JFS* so that you can see at a glance the "latest" information on the "hottest" subjects. These changes have been included in the latest version of the "*Journal of Food Science Style Guide for Research Papers*," which appears in this issue. Please follow it carefully when preparing your manuscript. Note that changes have also been made in the References section, requiring both the beginning and ending page numbers on cited references.

AS ALWAYS, the ultimate improvements in quality and subject matter depend on the authors and reviewers. Those of you who have served as reviewers during the past year are listed on the following pages. We thank you for your help. We also thank the authors who have chosen *JFS* as the publication vehicle for their manuscripts, and we invite all of you — authors, reviewers, and readers — to send us your suggestions, criticisms, or comments which can help the JFS editorial staff be more useful to you and your profession.





—Robert E. Berry, Scientific Editor,
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Sensory, Instrumental Texture Profile and Cooking Properties of Restructured Beef Steaks Made with Various Binders

C.M. CHEN and G.R. TROUT

ABSTRACT

Restructured steaks made with various binders were evaluated using subjective and objective texture profile analysis of the following treatments: intact ribeye muscle, calcium alginate, salt/tripolyphosphate, crude myosin extract, whey protein, wheat gluten, soy protein isolate, surimi and no additives. Steaks made with calcium alginate or crude myosin extracts had superior binding. Steaks with 1.5% surimi had similar textural properties to those with calcium alginate or crude myosin extract. Whey, wheat gluten or soy isolate protein in restructured steaks detrimentally affected product flavor. Calcium alginate, crude myosin extract and surimi could be potential binders in the manufacture of restructured steaks without deterimental effects on quality.

Key Words: Beef, texture, sensory evaluation, restructured meat, binders

INTRODUCTION

SALT AND PHOSPHATE are currently used in the manufacture of restructured meat products because of their beneficial effects on texture, yield and flavor (Schnell et al., 1970; Mandigo et al., 1972; Schmidt and Trout, 1982). However, salt is associated with discoloration of fresh restructured meat products (Chu et al., 1987), rancidity development (Huffman and Cordray, 1979; Trout and Schmidt, 1987) and may be a contributor to hypertension in susceptible segments of the population (Tobian, 1979; Pearson and Wolzak, 1982). Partial or complete replacement of salt with binders is a possible means of maintaining, and/or improving, quality of restructured beef steaks.

Proteins derived from a variety of plant and animal sources are used by the meat industry to reduce product cost and improve functionality. Soy protein (SP), whey protein (WP), and vital wheat gluten (WG) are nonmeat proteins commonly used. Many studies have evaluated the effect of those proteins as binders, fillers and extenders in various meat systems. These include production of comminuted and emulsified products, ground beef and beef patties, and restructured poultry products (Parks and Carpenter, 1987; Proteous and Quinn, 1979; Terrell et al., 1982). However, limited information is available regarding use of nonmeat proteins as binders in restructured steaks (Miller et al., 1986; Seideman et al., 1982; Siegel et al., 1979; Hand et al., 1981). Previous research with nonmeat proteins as binders in restructured steaks has been reported separately, and comparisons between different studies are difficult to interpret.

In addition other proteins and carbohydrates have potential value as binders in restructured meat products. Combinations of sodium alginate and calcium carbonate have been used as efficient binders in both raw and cooked restructured steaks (Means and Schmidt, 1986; Means et al., 1987). Also, crude myosin extracted from beef muscle was an effective binder in restructured beef steaks (Ford et al., 1978). Surimi, a product from fish muscle, may also be effective due to its unique binding ability (Burgarella et al., 1985).

There are, however, textural problems associated with restructured meat products manufactured using current technology. These include excessive or insufficient protein binding, distortion of the product during cooking, connective tissue residue, crust formation on the surface of cooked product, separation, layering and/or pocket formation in the cooked product, and nonuniform texture (Berry, 1987). Research on the use of various binders in restructured meat products and their influence on textural properties is limited. Hence, our research objective was to compare the textural properties of restructured beef steaks made with various binders to those of intact muscle beef steak.

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MATERIALS & METHODS

Meat and additives

Top rounds (semimembranosus) (cap off) and boneless ribeyes (longissimus dorsi) from USDA Choice beef carcasses were used for the products. Top rounds were obtained (John Morrell & Co., Montgomery, AL) 24 hr postmortem for each of three replications and trimmed of all visible fat and connective tissue. For the intact steak treatment, whole sections (longissimus dorsi) were removed from the boneless ribeye (24 hr postmortem) and trimmed to about the same sizes as the diam of casings. For restructured treatments, semimembranosus muscles were ground through a 3-hole kidney plate (2.5 × 6.0 cm^2) with a four-blade knife grinder to form meat chunks.

Additives used for preparation of treatments included reagent grade sodium chloride and calcium carbonate (Fisher Scientific Co., Fair Lawn, NJ), food grade sodium tripolyphosphate (FMC Corporation, Philadelphia, PA), sodium alginate (Manugel DMB, Kelco, Clark, NJ), encapsulated lactic acid (LCL-135-50, Balchem Co., Slate Hill, NY), whey protein concentrate (Alacen 882, New Zealand Milk Products, Inc., Petaluma, CA), isolated soy protein (Protein Technologies International, St. Louis, MO), wheat gluten (Supergluten-75, Ogilvie Mills, Inc., Minnetonka, MN), surimi (Alaska Fisheries Association, AK) and deionized water. Extracted beef crude myosin was prepared by the method of Turner et al. (1979).

Product preparation

The nine treatments are summarized in Table 1. The experiment was replicated three times. Restructured steaks were prepared by mixing meat chunks for 5 min (except salt and phosphate treatment) in a Hobart paddle type mixer (Model H-120, Hobart Co., Troy, OH) on speed setting 2 (200 rpm). Preliminary work (Trout and Chen, 1989)

Table 1—Treatment formulations and preparation procedures

Trt code	Treatment	Binder (%)	Mixing time (min)	Water (%)	NaCl (%)	STP (%)
IR	Intact muscle	–	–	–	–	–
CA	Calcium carbonate	0.1				
	Sodium alginate	0.5				
	Encapsulated lactate	0.5	5	7.0	–	–
SP	Salt/STP	–	3	7.0	1.00	0.50
CM	Crude myosin[a]	8.5	5	(7.0)	0.13	0.05
WP	Whey protein	2.0	5	7.0	0.13	0.05
WG	Wheat gluten	2.0	5	7.0	0.13	0.05
SI	Soy protein isolate	2.0	5	7.0	0.13	0.05
SU	Surimi	1.5	5	7.0	0.13	0.05
RC	Reconstructured control	–	5	7.0	–	–

[a] Protein content of crude myosin extract was 6%.
Final crude myosin content in the product was 0.5%.

in the manufacture of restructured steaks with salt and phosphate indicated mixing meat chunks for 5 min caused loss of steak like texture on the final product. Therefore, the mixing time for the salt and phosphate treatment was reduced to 3 min. During mixing, nonmeat ingredients and binders were added in accordance with the formulations shown in Table 1. The blended material from each treatment was stuffed into 1.5-cm-diameter (for instrumental trials) or 12.5-cm-diameter polyethylene casings (for sensory trials). With intact muscle treatment, the whole muscle sections were stuffed into casings as previously described for restructured products.

All logs except the calcium alginate (CA) treatment were frozen immediately after preparation at −34°C for 48 hr. The CA treatment was stored at 4°C for 24 hr and then frozen at −34°C for 24 hr. Forty-eight hr after preparation, each 10.5-cm log was sliced into 2-cm steakettes using a bandsaw with a stainless steel blade. Each steak was wrapped with oxygen permeable plastic interleaf sheets (Super Un-Lox, Phillips Petroleum Co., Chicago, IL), placed in E-Z Pak poly meat bags (2 Mil, low-density, freezing resist) and stored at −23°C for further analysis. The 12.5-cm logs were vacuum packaged and stored at −80°C for about 1 month and then sliced into 1.5-cm steakettes for sensory evaluation.

Cooking procedures

Steaks were removed from the freezer and held at room temperature for about 30 min before cooking. Weight and dimensional measurements before cooking were made during this time. Steaks were cooked from the frozen state on a Model TMM-36 Special McDonald's grill (Wolf Range Corporation, Compton, CA) at 165°C. Cooper constantan thermocouples were inserted into the approximate geometric center of each steak to monitor internal temperature. Steaks were cooked on each side for 3 min alternately to internal temperature of 70°C (total cooking time was 12 min for 2-cm steakettes and 9 min for 1.5-cm steakettes).

Cooking loss and dimensional changes

Steaks were weighed and thickness and diam measured before and after cooking to determine cooking losses and dimensional changes. The diam and thickness of frozen and cooked steaks was measured with a micrometer (Marostat, Bern, Switzerland) at 3 random locations and expressed as percentage changes.

Cooking losses were determined as follows:

$$\text{Cooking loss (\%)} = \frac{\text{Raw wt} - \text{Cooked wt}}{\text{Raw wt}} \times 100$$

Percentage changes in dimensions were calculated as follows:

$$\text{Diameter change (\%)} = \frac{\text{Raw diam} - \text{Cooked diam}}{\text{Raw diam}} \times 100$$

$$\text{Thickness change (\%)} = \frac{\text{Raw thickness} - \text{Cooked thickness}}{\text{Raw thickness}} \times 100$$

Sensory texture profile evaluation

Texture of restructured beef steaks was evaluated by a 13-member trained sensory panel. Panel training was conducted during several sessions according to procedures described by Civille and Szczesniak (9173). Procedures and textural parameters used for panel evaluation were adapted from those described by Berry (1987) and Brady et al. (1985). Steaks (1.5 cm thick) were cooked as previously described and then cut into 2-cm squares. Three treatments were served in every section to panel members. Panel members evaluated samples for springiness, cohesiveness, hardness, moisture release, denseness, separation of chunks, chewiness, cohesiveness of mass, juiciness, adhesiveness, mealiness, mouth coating and nonmeat flavor on an eight-point scale. Definitions and procedures are described in Table 2.

Instrumental texture profile

The procedure used for instrumental texture profile analysis was similar to those described by Bourne (1982) and Brady et al. (1985). Two frozen steaks per treatment were cooked as previously described and texture profile was measured with an Instron Universal Testing Machine (Model 1011, Instron Corp., Canton, MA). Four 2.5-cm squares were removed from the center portion of each cooked steak and compressed to 50% of original height. A 50-kg load cell was used with a load range of 0–50 kg at crosshead and chart speeds of 200 mm/min. Two compression cycles per slice were generated to form a "two-bite" work-force compression curve. The textural parameters derived from the two successive compression curves were hardness, cohesiveness, springiness, gumminess and chewiness (Bourne, 1968).

Table 2—Texture profile panel attributes, procedures and description for restructured beef steaks

I.	Partial compression:	
		Place a warm, 4-cm² piece in the mouth, using the molar against the cooked surfaces, press lightly five times. Wait 2 sec between presses.
		Springiness—The perceived degree and speed with which the sample returns to the original height and thickness after pressing five times.
II.	First bite:	
		Take a warm, 4.0-cm² piece and place it in the mouth and, using the molars against the cooked surfaces, make the first incision and evaluate for:
		Cohesiveness—The degree to which the sample de forms before shearing.
		Hardness—Amount of force required to bite through sample.
		Moisture release—Amount of juiciness perceived dur ing the first bite.
		Compactness—Degree the meat particles are crowded together.
III.	Mastication:	
		Take a warm, 4.0-cm² sample, make the first incision as for first bite. Then turn the two pieces 90 degrees and take a second bite. Evaluate for:
		Chunk separation—Check the appropriate breakdown category.
		Continue chewing and count the number of chews then evaluate for:
		Chewiness—Number of chews required to prepare sample for swallowing.
		Cohesiveness of mass—The degree to which meat chunks bind together.
		Juiciness—The amount of juice released.
		Adhesiveness—Degree to which sample sticks to mouth.
		Mealiness—The degree of powdery or hamburger-like pieces perceived during chewing.
IV.	After swallowing	
		Mouth coating—Amount of film residue left on mouth surface after swallowing.
		Nonmeat flavor—Amount of nonmeat flavor left in the mouth after swallowing.

Warner-Bratzler shear force

Steaks were cooked as previously described and cut into 2.5-cm squares. Eight squares from each treatment were sheared using a Warner-Bratzler shear cell attachment on the Instron Universal Testing Machine (Model 1011, Instron Corp., Canton, MA). Samples were placed perpendicular to the shear blade and sheared once. A 50-kg load cell with a load range of 0–50 kg was used and crosshead and chart speeds were set at 200 mm/min. Shear force values were reported as kg per 6.25 cm².

Tensile strength measurement

Steaks were cooked as previously described and cut into slices (1.5 cm wide × 0.8 cm thick) by hand with a templet. Two cooked steaks of each treatment (8–10 slices) were subjected to measurement of tensile strength with a modified Warner-Bratzler Meat Shear Machine (Model 2000, G.R. Electric Mfg. Co., Manhattan, KS) (Trout and Schmidt, 1984). Tensile strength was expressed as g/cm² cross-sectional area.

Statistical analysis

Data were analyzed using a completely randomized block design with 9 treatments and 3 replications. For sensory evaluation, an incomplete block design (partial lattice design) was used to eliminate the variation of experimental units within an incomplete block (Cochran and Cox, 1957). Analysis of variance, means and standard errors were computed using the Statistical Analysis System (SAS, 1982).

Table 3—Sensory texture profile analysis of restructured beef steaks

Trt[a]	Sensory parameters[f,g]							
	SPRG	HARD	MORE	MSCO	JUIC	MEAL	MOCO	NOMF
IR	4.47^{c}	4.98^{ab}	5.84^{ab}	5.90^{a}	6.17^{ab}	3.33^{d}	2.46^{d}	2.53^{d}
CA	5.19^{bc}	4.10^{c}	4.84^{c}	5.22^{ab}	4.87^{cd}	4.00^{c}	2.76^{bcd}	2.84^{cd}
SP	6.41^{a}	5.31^{a}	6.72^{a}	5.83^{ab}	6.78^{a}	3.45^{d}	2.74^{bcd}	3.13^{bcd}
CM	5.91^{ab}	5.38^{a}	5.44^{bc}	5.60^{ab}	5.46^{bc}	3.54^{d}	2.67^{cd}	2.77^{d}
WP	4.90^{bc}	5.07^{ab}	5.64^{bc}	5.00^{ab}	5.39^{bcd}	4.36^{abc}	3.02^{ab}	3.72^{bc}
WG	5.74^{ab}	4.51^{bc}	5.36^{bc}	4.94^{b}	5.19^{cd}	4.54^{ab}	2.91^{abc}	3.87^{b}
SI	5.65^{ab}	4.62^{bc}	5.25^{bc}	4.95^{ab}	5.08^{cd}	4.74^{a}	3.17^{a}	4.79^{a}
SU	5.63^{ab}	4.82^{ab}	5.14^{bc}	5.10^{ab}	5.00^{cd}	4.74^{a}	2.78^{bcd}	2.90^{cd}
RC	5.76^{ab}	4.97^{ab}	4.93^{bc}	5.07^{ab}	4.57^{d}	4.17^{bc}	2.56^{d}	2.61^{d}
SEM	0.26	0.09	0.20	0.10	0.15	0.05	0.03	0.10

[abcd] Means in the same row with different superscripts are different ($P<0.05$).

[a] Treatment: see Table 1 for definitions

[f] Evaluation on an 8-point scale (1 = extremely nonspringy, extremely soft, extremely dry, extremely noncohesive, extremely dry, devoid, none, none; 8 = extremely springy, extremely hard, extremely juicy, extremely cohesive, extremely juicy, extremely abundant, extremely coated, extremely intense).

[g] Sensory parameter: SPRG = springiness; HARD = hardness; MORE = moisture release; MSCO = mass cohesiveness; JUIC = juiciness; MEAL = mealiness; MOCO = mouth coating; NOMF = nonmeat flavor.

Fisher's Least Significant Difference test was used to determine differences between treatment means (Steel and Torrie, 1980) when analysis of variance indicated a significant effect at $P<0.05$ level.

RESULTS & DISCUSSION

Sensory texture profile

There were no differences ($P<0.05$) between treatments for cohesiveness, compactness, separation of meat chunks, chewiness or adhesiveness (data not shown). However, there were differences ($P<0.05$) between treatments for springiness, hardness, moisture release, cohesiveness of mass, juiciness, mealiness, mouth coating and nonmeat flavor (Table 3).

Springiness scores (during the first bite) were lower ($P<0.05$) for intact ribeye (IR) steaks than for steaks from all other treatments, except restructured steaks made with CA and WP. Restructured streaks made with CA had lower ($P<0.05$) hardness scores than all other treatments except for restructured steaks made with WG or SI. Restructured steaks from all treatments (except calcium alginate) had similar hardness scores to IR. There was greater ($P<0.05$) moisture release from steaks made with NaCl and STP (SP) than from all other steaks except for IR. No differences ($P>0.05$) were found among the remaining treatments for moisture release scores.

Restructured steaks produced with WG had lower ($P<0.05$) cohesiveness of mass scores (determined during mastication) than IR. There was no difference ($P<0.05$) for cohesiveness of mass scores between IR and restructured steaks made with CA, SP, crude myosin extract (CM), WP, SI, surimi (SU), or restructured control (C).

Sensory panel scores for juiciness (determined during mastication) followed similar trends as the results for moisture release. Panelists rate IR and restructured steaks made with SP higher ($P<0.05$) for juiciness than steaks containing CA, WG, SI, SU or the restructured control. Restructured control (no additives) had a lower ($P<0.05$) juiciness score than IR steaks and restructured steaks made with SP, or CM. Steaks containing SP, CM, or WP had similar juiciness scores to the IR steaks. This indicated that SP increased water-holding capacity of the restructured steaks, which was in agreement with earlier reports of Huffman et al. (1981) and Hand et al. (1981).

IR steaks and the restructured steaks with SP or CM were less ($P<0.05$) mealy during mastication than the other steaks. Additionally, restructured steaks made with SI or SU were more mealy ($P<0.05$) than those from other treatments except those made with WP or WG.

The restructured steaks produced with SI had more pronounced ($P<0.05$) mouth coating than the other steaks, except the restructured steaks made with WP or WG. No differences ($P>0.05$) were found for mouth coating between steaks produced with CA, SP, CM, SU, or C and IR steaks. A stronger ($P<0.05$) nonmeat flavor was detected in restructured steaks made with SI than steaks from all other treatments. Steaks made with ST, CA, CM, or SU had similar mouth coating and nonmeat flavor scores to the IR steaks and C. Results of sensory texture profile evaluation for mouth coating and nonmeat flavor were similar to those reported by Seideman et al. (1982) and Hand et al. (1981). Seideman et al. (1982) found that restructured steaks made with 2% WG had rancid, bitter and other off-flavors. Hand et al. (1981) reported that restructured steaks made with WG were less detrimental to off-flavor than steaks made with SI.

Table 4—Instrumental texture profile analysis of restructured beef steaks[a]

Trt[f]	Hardness	Cohesiveness	Springiness	Gumminess	Chewiness
IR	9.78^{bcd}	0.31^{d}	0.49^{d}	2.84^{d}	1.31^{e}
CA	7.98^{d}	0.40^{d}	0.67^{c}	3.12^{d}	2.00^{de}
SP	12.47^{b}	0.39^{c}	0.85^{b}	4.89^{bc}	4.13^{b}
CM	12.50^{b}	0.44^{bc}	0.71^{c}	5.33^{bc}	3.73^{b}
WP	9.17^{cd}	0.44^{bc}	0.68^{c}	3.95^{cd}	2.67^{cd}
WG	11.91^{b}	0.41^{bc}	0.70^{c}	4.93^{bc}	3.48^{bc}
SI	12.09^{b}	0.46^{bc}	0.74^{c}	5.66^{b}	4.11^{b}
SU	10.83^{bc}	0.46^{bc}	0.66^{c}	5.01^{bc}	3.30^{bc}
RC	10.04^{bcd}	0.48^{b}	0.67^{c}	4.84^{bc}	3.15^{bc}
SEM	0.35	0.01	0.01	0.19	0.14

[a] Hardness = peak force during first compression cycle; cohesiveness ratio of force area during second compression to that during first compression; springiness = height the meat recovers during the time that elapses between end of fist bite and start of second bite; gumminess = product of hardness × cohesiveness; chewiness = product of gumminess × springiness.

[bcde] Means in the same column with a different superscript are different ($P<0.05$).

[f] Treatment: see Table 1 for definitions

Instrumental texture profile analysis

There were differences ($P<0.05$) between steaks for instrumental hardness, cohesiveness, springiness, gumminess and chewiness values (Table 4). Steaks with CM, SP, WG, or SI were harder ($P<0.05$) than those made with CA or WP. Steaks with CA had lower ($P<0.05$) scores for hardness than those from all other treatments except IR steaks and restructured steaks with WP or no additives.

IR steaks had the lowest ($P<0.05$) score for cohesiveness. Restructured control had the higher ($P<0.05$) cohesiveness scores than IR steak and restructured steaks with CA or SP. Ony minor differences occurred in cohesiveness between steaks from other treatments, except IR steak and C. Springiness was greatest ($P<0.05$) forsteaks with SP and least for IR steaks. For gumminess, the IR steaks and the restructured steaks with CA had lower ($P<0.05$) values than those from other treatments except for the restructured steaks made with WP. No significant difference ($P>0.05$) was observed for gumminess between steaks from the remaining treatments. Steaks prepared with CA had similar chewiness values to IR steaks which had the lowest chewiness scores. Steaks with SP, CM extract, or SI had higher ($P<0.05$) chewiness values than those from other treatments, except C and steaks with WG and SU.

Table 5—Physical and cooking properties of restructured beef steaks

Trt[e]	Tensile strength (g/cm^2)	Cook loss (%)	Dimensional changes (%)	
			Diameter	Thickness
IR	303[a]	17.3[cd]	−15.2[a]	−11.3[a]
CA	204[bc]	15.6[d]	−12.6[a]	−4.9[a]
SP	236[b]	17.5[cd]	−15.7[a]	+8.7[b]
CM	183[cd]	25.5[ab]	−17.0[a]	−5.9[a]
WP	145[d]	21.4[bc]	−14.9[a]	−6.9[a]
WG	185[cd]	25.4[ab]	−15.9[a]	−7.2[a]
SI	162[cd]	24.3[ab]	−16.1[a]	−6.3[a]
SU	168[cc]	25.5[ab]	−15.2[a]	−9.4[a]
RC	154[cd]	28.8[a]	−17.3[a]	−8.6[a]
SEM	60	8.3	3.0	18.8

[abcd] Means in the same column with a different superscript are different ($P<0.05$).
[e] Treatment: see Table 1 for definitions.

Warner-Bratzler shear force and tensile strength

Warner-Bratzler shear force values were not affected ($P>0.05$) by the different binders or processing procedures. The mean value for the shear force measurement of all treatments was 3.54 kg/per 6.25 cm^2 area (SEM = 0.71). Tensile strength values were different ($P<0.05$) between steaks. IR steaks had the highest tensile strength values. Steaks containing SP had higher ($P<0.05$) tensile strength values than all other treatments except the IR steak and those with CA. These results agreed with those reported by Huffman et al. (1981) and Trout and Schmidt (1984), who found that salt and phosphate improved binding strength due to greater extraction of salt soluble proteins. Restructured steaks made with different binders (CM, WG, SI, or SU) had similar tensile strength values to those with CA except for restructured steaks with WP.

Cooking loss

Results for cooking loss and dimensional changes for steaks are presented in Table 5. IR steaks and restructured steaks produced with CA or SP had lower $P<0.05$) cooking loss than the other restructured steaks except for steaks with WP. This observation for steaks made with CA was compatible with the work of Clarke et al. (1988) who found reduced cook loss in restructured beef due to a combination of slightly elevated pH and inhibition of moisture migration by CA. Trout and Schmidt (1984) also reported that the addition of salt and phosphate increased cooking yield due to an increase in ionic strength and pH. Steaks made with WI had similar ($P>0.05$) cooking losses to IR steaks and SP steaks. No differences ($P>0.05$) were observed for cooking loss among steaks from treatments with binders such as CM, WG, SI or SU.

The diameter of all steaks decreased after cooking and ranged from 12.6 to 17.3%. However, these decreases were not affected ($P>0.05$) by the binders. Thickness of all steaks decreased after cooking except those produced with SP, which increased in thickness by 8.7%. There were no other differences ($P>0.05$) for thickness changes of steaks among other treatments.

CONCLUSIONS

Results indicate several binders have potential use in the manufacture of low-salt restructured beef steaks. Those with CA or CM had superior binding ability and moisture retention, as determined by sensory texture profile evaluation, instrumental analysis and tensile strength measurements. SU could be as good a binder as CM or CA. However, steaks made with SU had greater cooking losses. The use of WP, WG, or SI as binders in restructured beef steaks may detrimentally affect flavor. CA, CM, or SU could be potential binders in low-salt restructured beef steaks without notably detrimental effects of quality.

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Color and Its Stability in Restructured Beef Steaks during Frozen Storage: Effects of Various Binders

C.M. CHEN and G.R. TROUT

ABSTRACT

Color and its stability were evaluated in restructured steaks made with various binders (calcium alginate, crude myosin extract, whey protein concentrate, wheat gluten, soy isolate or surimi) vs controls (intact ribeye muscle, restructured steaks with no additives or with NaCl and sodium tripolyphosphate). Steaks made with various binders showed similar effects on initial surface metmyoglobin concentration and sensory color attributes, except steaks made with calcium alginate or soy isolate protein. During 12-wk frozen storage, steaks made with various binders (except soy protein isolate) had similar color stabilities.

Key Words: binders, beef, color, restructured steaks, frozen storage

INTRODUCTION

THE USE OF SALT and phosphate is important in the manufacture of restructured products because of beneficial effects on myofibrillar protein extractability and a subsequent increase in binding (Schnell et al., 1970), yield and flavor (Mandigo et al., 1972; Huffman et al., 1981; Schmidt and Trout, 1982). However, salt has been also associated with discoloration of fresh restructured products (Chu et al., 1987; Booren and Mandigo, 1981); rancidity development (Huffman and Cordray, 1979; Trout and Schmidt, 1987) and as a contributor to hypertension in susceptible consumers (Tobian, 1979; Pearson and Wolzak, 1982). Thus, there is a need to lower sodium chloride levels in meat products.

The simple reduction of currently used salt levels would appear to be the most efficient method to develop low-salt meat products. However, this results in products with less desirable textural and palatability traits. Olson and Terrell (1981) reported the flavor and texture of meat products with salt substitutes such as potassium chloride and magnesium chloride was inferior to that of products manufactured with sodium chloride. Therefore, partial replacement of salt with binders is a possible means of maintaining, or improving, those properties of restructured beef steaks.

The use of exogenous proteins or binders is of great interest for the meat industry to produce high quality, economical meat products. Soy protein, whey protein and vital wheat gluten are three nonmeat proteins most commonly used in meat products. Many studies have evaluated their effects as binders, fillers and extenders in various meat systems (Proteous and Quinn, 1979; Hand et al., 1981; Terrell et al., 1982; Parks and Carpenter, 1987). In addition to those proteins, several other proteins or compounds have potential value as binders in restructured meat products. Combinations of sodium alginate and calcium carbonate have been used as efficient binders both in raw and cooked restructured steaks (Means and Schmidt, 1986; Means et al., 1987). Crude myosin extracted from beef muscle was also an effective binder in restructured beef steaks (Ford et al., 1978). Surimi (manufactured from fish muscle) may also be used due to its unique binding ability (Burgarella et al., 1985).

Appearance and color of meat products are prime factors by which consumers judge their acceptability. Discoloration is a major problem for marketing restructured meat products since brown spots among the red-bloom meat color decrease consumer acceptability. Hood and Riordan (1973) reported a linear increase in discrimination by consumers against discolored meat as the percentage of metmyoglobin increased in fresh meat.

The objective of our study was to investigate color and color stability of restructured beef steaks made with various binders during frozen storage.

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MATERIALS & METHODS

Meat and additives

Top rounds (USDA Choice) were obtained 24 hr postmortem from a commercial operation for each of 3 replications and were trimmed of all visible fat and connective tissue. For the intact steak treatment, whole sections (longissimus dorsi) were removed from the boneless ribeye (USDA Choice) and trimmed to about the same size as the diameter of casings (10.5-cm-diameter). For restructured treatments, top rounds (semimembranosus) were ground through a 3-hole kidney plate (2.5 × 6.0 cm^2) to form meat chunks.

Additives used for reference control treatments were reagent grade sodium chloride (Fisher Scientific Co., Fair Lawn, NJ), food grade sodium tripolyphosphate (FMC Corporation, Philadelphia, PA). Additives for calcium alginate treatment consisted of 0.1% calcium carbonate (Fisher Scientific Co., Fair Lawn, NJ), 0.5% sodium alginate (Manugel DMB, Kelco, Division of Merck and Company, Inc., Clark, NJ) and 0.5% encapsulated lactic acid (LCL-135-50, Balchem Co., Slate Hill, NY). Various binders included whey protein concentrate (Alacen 882, New Zealand Milk Products, Inc., Petaluma, CA), isolated soy protein (Protein Technologies International, St. Louis, MO), wheat gluten (Supergluten-75, Ogilvie Mills, Inc., Minnetonka, MN) or surimi (Alaska Fisheries Association, Kodiak, AK). Extracted beef crude myosin was prepared by the method of Turner et al. (1979).

Product preparation

The 9 treatments are summarized in Table 1. The experiment was replicated 3×. Restructured steaks were prepared by mixing meat chunks for 5 min (except salt and phosphate treatment, 3 min) in a Hobart paddle type mixer (Model H-120, Hobart Co., Troy, OH) on speed setting 2 (200 rpm) at 5°C. Preliminary work (Trout and Chen, 1989) indicated that the mixing time for restructured steaks made with

Table 1—Treatment formulations and preparation procedures

Trt	Treatment	Binder (%)	Mixing time (min)	Water (%)	NaCl (%)	STP (%)
IR	Intact ribeye	–	–	–	–	–
CA	Calcium carbonate	0.1				
	Sodium alginate	0.5				
	Encapsulated lactate	0.5	5	7.0	–	–
SP	Salt & STP	–	3	7.0	1.00	0.50
CM	Crude myosin[a]	8.5	5	(7.0)	0.13	0.05
WP	Whey protein	2.0	5	7.0	0.13	0.05
WG	Wheat gluten	2.0	5	7.0	0.13	0.05
SI	Soy isolate	1.5	5	7.0	0.13	0.05
SU	Surimi	1.5	5	7.0	0.13	0.05
RC	Restructured control	–	5	7.0	–	–

[a] Protein content of crude myosin extract was 6%. Final crude myosin content in the product was 0.5%.

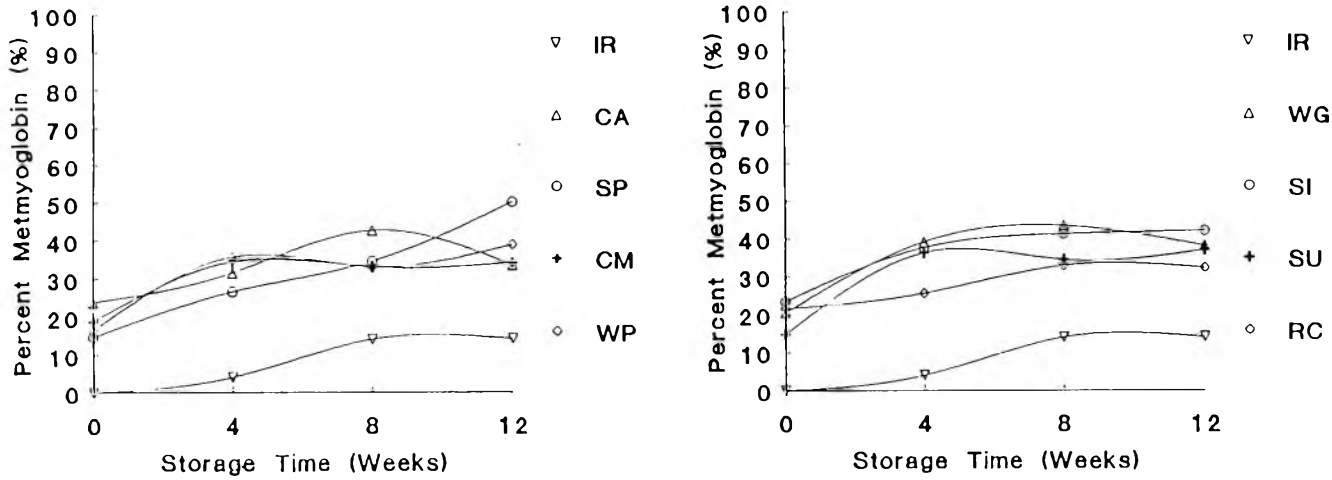

Fig. 1—Effects of various binders on percent metmyoglobin concentrations (by absorbance) of restructured beef steaks after 12 wk storage: IR: Intact ribeye steaks; CA: calcium alginate; SP: NaCl & STP; CM: crude myosin extracts; WP: whey protein; WG: wheat gluten; SI: soy isolate; SU: surimi; RC: restructured control.

salt and phosphate should be kept to <3 min. Excessive mixing (>3 min) of meat chunks caused loss of steaklike texture in the final product.

During mixing, nonmeat ingredients and binders were added as indicated (Table 1). For the surimi treatment, the mixture was made with "activated" surimi. The surimi activation process consisted of chopping frozen surimi with 0.8% salt and 1.5% phosphate (wt of ingredients/wt of surimi block) in a Presto Minnie Compact Food Processor (National Presto Ind., Inc., Eau Claire, WI) for 30 sec. The blended material from each treatment was stuffed into a presoaked, prestuck, 10.5-cm-diameter polyethylene casing using a Dick piston valve stuffer (Hubert Co., Cincinnati, OH). For the intact treatment, the whole muscle sections were stuffed into casings as previously described for restructured products.

All logs except the calcium alginate treatment were frozen immediately after preparation at −34°C for 48 hr. The calcium alginate treatment was stored at 4°C for 24 hr (to allow cross-linked network of polymer chains to form) and then frozen at −34°C for 24 hr according to Trout et al. (1990). The procedures used were determined to be optimum for each treatment. Forty-eight hr after preparation, each log was sliced into 2-cm steakettes using a bandsaw with a stainless steel blade. Each steak was wrapped with an oxygen permeable plastic interleaf sheet (Super Un-Lox, Phillips Petroleum Co., Chicago, IL), placed in a plastic bag and stored at −23°C for further analyses. All operations were conducted at 4°C to minimize color changes. Steaks were randomly selected for analysis initially and over storage periods of 4, 8, and 12 wk.

Surface metmyoglobin concentration

Surface percent metmyoglobin analysis by absorbance was determined by the procedure of Trout et al. (1990). Steaks from each treatment were held at room temperature for 5 min before analysis. Three 5-g samples were removed from both surfaces of the steak by cutting slices about 1-mm thick. Myoglobin was extracted with cold 0.04M phosphate buffer, pH 6.8 using sample: buffer = 1:10. Samples were homogenized for 30 sec on speed setting 4 (10,800 rpm) with a Polytron homogenizer (Brinkmann homogenizer, Westbury, NY). The homogenates were then centrifuged for 30 min (50,000 × *g*) at 5°C. The absorbance of filtered supernatant was measured at 525, 572 and 730 nm using a Perkin-Elmer Lambda 4A spectrophotometer (Norwalk, CT). The percent metmyoglobin was determined using the following formula described by Krzywicki (1979) with the turbidity correction suggested by Goldbloom and Brown (1966):

$$\text{Metmyoglobin (\%)} = \left(1.395 - \frac{A572 - (A730 \times 1.45)}{A525 - (A730 \times 1.73)}\right) \times 100$$

where A = absorbance at nm.

Reflectance surface metmyoglobin concentration

Percent surface metmyoglobin concentration was measured with a Perkin-Elmer Lambda 4A spectrophotometer (Norwalk, CT) equipped with a standard reflectance attachment. The steak was covered with PVC film to prevent meat drippings from entering the integrating sphere. The PVC film exhibited no measurable absorption of light between 380 nm and 760 nm. The instrument was calibrated to 100% reflectance with a standard reference material (magnesium oxide) that was covered with PVC film. The absorbance of samples was scanned from 380 to 760 nm at 4 random locations. Percent metmyoglobin was calculated as described by Krzywicki (1979). CIE L,a,b, values were also calculated from the reflectance spectra.

Sensory color evaluation

Surface discoloration and overall color appraisal of steaks were evaluated by a 10-member trained sensory panel. Steaks were removed from the freezer, unwrapped, covered with interleaf paper, held at 4°C for 4 hr and evaluated under warm white fluorescent lighting that provided 70 ft-c at the counter surface. Panel members rated each steak on an 8-point scale, with one = 100% discoloration or extremely brown and 8 = 0% discoloration or extremely red for surface discoloration and overall color, respectively.

Statistical analyses

This study was analyzed using a split-plot design with 9 treatments and 3 replications. Analysis of variance, means and standard errors were computed using the Statistical Analysis System (SAS, 1982). Significant treatment means were separated using Fisher's Least Significant Difference (FLSD) test to detect differences between treatment means (Steel and Torrie, 1980) when analysis of variance indicated a significant effect.

RESULTS & DISCUSSION

Percent surface metmyoglobin-absorbance

Differences ($P<0.01$) were observed among treatments in surface metmyoglobin concentration over 12-wk frozen storage (Fig. 1). The initial surface metmyoglobin concentration of intact ribeye steaks was the lowest ($P<0.01$) of all treatments. Restructured steaks made from NaCl + STP, crude myosin extract, whey protein or surimi had lower ($P<0.05$) initial surface metmyoglobin concentration than restructured steaks made from calcium alginate or soy isolate protein.

Surface metmyoglobin concentration of intact ribeye steaks remained the lowest over 12-wk storage. There were no differences ($P>0.05$) between restructured steaks with no additives and those made from any of the following binders: calcium alginate, crude myosin extract, whey protein, wheat gluten or surimi at 12-wk frozen storage. Restructured steaks made from NaCl + STP or soy isolate protein showed higher ($P<0.05$) surface metmyoglobin concentration than other treatments.

The surface metmyoglobin concentration of products in-

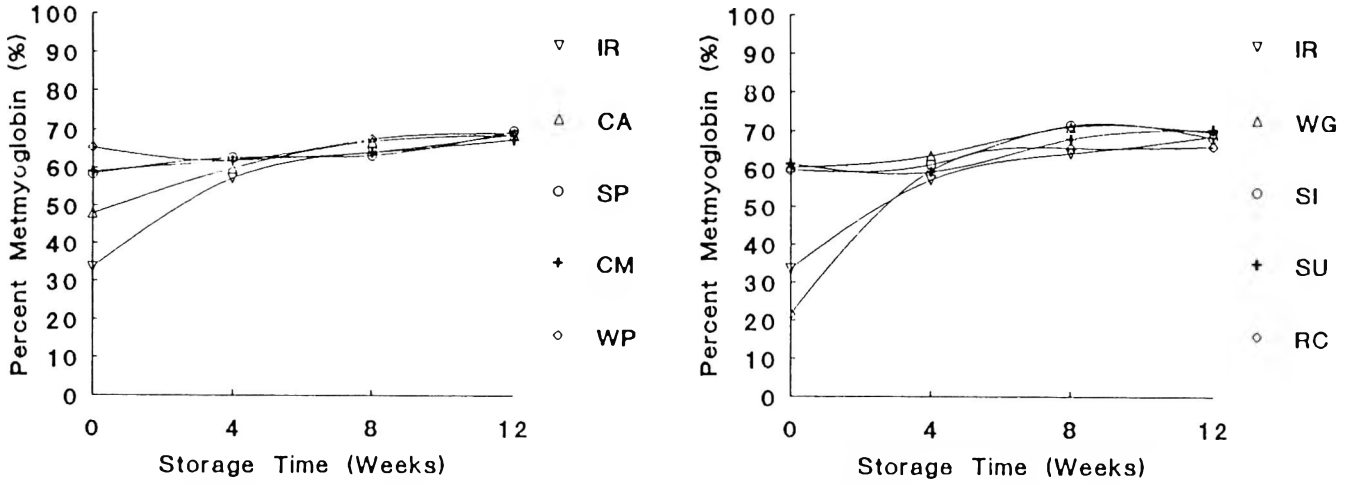

Fig. 2—Effects of various binders on percent metmyoglobin concentration (by reflectance) of restructured beef steaks after 12 wk storage: IR: Intact ribeye steaks; CA: calcium carbonate; SP: NaCl + STP; CM: crude myosin extracts; WP: whey protein; WG: wheat gluten; SI: soy isolate; SU: surimi; RC: restructured control.

creased ($P<0.01$) with storage time (Fig. 1). As was expected, restructured steaks made from NaCl + STP had a rapid increase ($P<0.01$) in surface metmyoglobin concentration over 12 wk frozen storage. The results were in agreement with the finding of Chu et al. (1987) who reported that increased myoglobin oxidation rate was induced by the addition of sodium chloride. This salt did not increase initial overall metmyoglobin but increased the rate of metmyoglobin formation. Rate of surface metmyoglobin formation was slower in the restructured control and restructured steaks made from calcium alginate or crude myosin extract over the storage period. Restructured steaks containing calcium alginate had higher ($P<0.05$) initial surface metmyoglobin concentration, but slower ($P<0.05$) metmyoglobin formation rates than other treatments during frozen storage. This was consistent with previous reports by Trout and Chen (1989) and Trout et al. (1990). Calcium carbonate apparently had a protective effect on myoglobin oxidation rate which could be due to its increase in pH or an anion or cation effect.

Tristimulus color L,a,b values

There were no differences ($P>0.01$) for tristimulus color "L" (lightness), "a" (redness) and "b" (yellowness) values due to the binders (data not presented). Mean values for "L", "a" and "b" were 43.8 ± 0.8, 9.2 ± 0.6 and 8.5 ± 1.0, respectively. However, "L" and "a" values were affected ($P<0.05$) by storage time. The "L" values for all treatments, in general, followed the same trend, decreasing at early stages of storage, and then increasing during the storage period from 8 to 12 wk. The changes in "a" values for all treatments over the storage period were variable. No differences ($P>0.01$) in "b" values were observed among treatments over storage time.

Percent surface metmyoglobin concentration-reflectance

There were differences ($P<0.01$) for surface reflectance metmyoglobin concentration among the treatments (Fig. 2). The initial metmyoglobin concentration was the lowest ($P<0.05$) for intact ribeye steaks and the highest ($P<0.05$) for restructured steaks made with whey protein. Restructured steaks made with calcium alginate were lower ($P<0.05$) for surface metmyoglobin concentration than those made with other binders.

At 12 wk storage, restructured steaks from surimi had the highest ($P<0.05$) surface metmyoglobin concentration of all treatments. This did not agree with results found from the absorbance data. Why a difference was found between the two methods is not clear. Further analysis of the data for surface metmyoglobin concentration indicated very low correlation between reflectance and sensory results ($r = -0.23$ for overall color score and $r = -0.28$ for discoloration score) or absorbance results ($r = 0.09$). The metmyoglobin concentration of steaks changed ($P<0.01$) with storage time. These changes were variable with treatments at different times (Fig. 2).

Table 2—Effects of various binders on overall color score[a] of restructured beef steaks during storage

	Storage time (wk)			
Trt[b]	0	4	8	12
IR	6.83	7.05	7.11	6.58
CA	4.53	4.77	4.54	5.10
SP	6.25	6.27	5.19	3.83
CM	5.37	4.63	4.52	5.03
WP	5.72	4.59	4.34	5.15
WG	5.50	4.63	4.23	4.82
SI	5.38	5.18	3.76	3.85
SU	5.41	4.45	5.03	4.40
RC	5.93	5.20	5.34	5.42

[a] Overall color score by trained sensory panel using 8-point scale (1 = extremely brown; 8 = extremely red).
[b] For treatments see Table 1.
[c] $LSD_{0.05}$ = 1.088 for comparison of means between treatment for a given storage time. $LSD_{0.05}$ = 0.371 for comparison of means by storage time within a given treatment.

Overall color panel scores

Panelists evaluating color found significant ($P<0.05$) differences between treatments for overall color score (Table 2). Intact ribeye steaks and restructured steaks made with NaCl + STP had the highest ($P<0.05$) initial overall color scores (more red). Restructured steaks made with calcium alginate had the lowest ($P<0.05$) initial overall scores (more brown). After 12 wk storage, restructured steaks made from NaCl + STP or soy protein isolate had the lowest ($P<0.05$) overall color scores. Intact ribeye steaks had the highest ($P<0.05$) overall color scores.

The overall color scores were affected ($P<0.01$) by storage time (Table 2). Overall color score decreased ($P<0.01$) over 12-wk frozen storage for all restructured steaks except intact ribeye steaks and restructured steaks made with calcium alginate. Intact ribeye steaks maintained the highest ($P<0.01$) overall color scores over 12-wk storage. Overall color score of restructured steaks made from calcium alginate increased ($P<0.01$) as storage time increased. These results were in agreement with the results for surface metmyoglobin concentration by the absorbance method.

Table 3—Effects of various binders on percent discoloration[a] score of restructured beef steaks during storage

	Storage time (wk)			
Trt[b]	0	4	8	12
IR	2.38	0.38	3.75	5.25
CA	42.13	31.88	36.63	26.50
SP	11.88	10.13	28.13	50.63
CM	24.25	37.50	36.88	30.25
WP	19.63	35.38	38.63	37.63
WG	24.00	34.50	41.88	32.38
SI	28.50	20.38	47.63	46.63
SU	21.88	32.63	29.50	38.75
RC	12.13	19.50	25.00	24.38

[a] Discoloration color score by trained sensory panel using 8-point scale (1 = 0% discoloration; 8 = 100% discoloration).
[b] For Treatments see Table 1.
[c] $LSD_{0.05}$ = 1.447 for comparison of means between treatment for a given storage time. $LSD_{0.05}$ = 0.412 for comparison of means by storage time within a given treatment.

Surface discoloration

Surface discoloration differences ($P<0.05$) occurred among restructured steaks containing various binders (Table 3). Initial surface discoloration scores (at 0 week) for intact ribeye steaks and restructured control were lower ($P<0.05$) (3 and 12% surface discoloration, respectively) than steaks from the other treatments. Restructured steaks that contained calcium alginate had greater ($P<0.05$) initial discoloration (around 42%) than other treatments. The initial surface discoloration for the other treatments ranged from 28.5 to 19.8%. After 12 wk frozen storage, intact ribeye steaks had less ($P<0.05$) discoloration (5.3%) than all other treatments. Restructured steaks containing NaCl + STP had the greatest ($P<0.05$) amount of discoloration (50%).

Surface discoloration changed ($P<0.01$) with storage time (Table 3). Discoloration of all treatments except calcium alginate increased ($P<0.05$) with storage time. However, the surface discoloration of restructured steaks made with calcium alginate decreased with storage time. Changes in surface discoloration over 12 wk storage in intact ribeye steaks, restructured steaks made with myosin extract or wheat gluten were small ($P<0.05$) when compared to the remaining treatments (2.9, 8.4 and 6.0% changes, respectively). Restructured steaks containing NaCl + STP had the greatest ($P<0.05$) increase in surface discoloration (decrease 38.8%) during frozen storage.

Results from the visual surface discoloration appraisal indicated that intact ribeye steaks, and restructured steaks containing crude myosin extract, calcium alginate, or wheat gluten had better color stability than steaks from other treatments. Restructured steaks containing NaCl + STP had lower ($P<0.05$) color stability than other treatments. Restructured steaks containing calcium alginate had the greatest ($P<0.05$) color stability of restructured steaks, although the reason for this was not clear.

The color of fresh meat is determined mainly by the relative proportions of three meat pigment derivatives: purple reduced myoglobin, red oxymyoglobin and brown metmyoglobin. It is a random distributed pigment system. Because of the complexity of color evaluations (visual or instrumental), numerous systems can be applied to measure color appearance of the meat product.

Hunter color and CIE-tristimulus methods were developed to approximate a physical description of the actual, perceived color of the meat (Hunt, 1980). Reflectance and transmission spectrophotometry are measurements of the radiant energy-transmission of a sample and relate directly to myoglobin properties in meats (Hunt, 1980).

In our study several different color measurements such as reflectance and transmission spectrophotometry, tristimulus colorimeters and visual evaluation were used. As expected, there was a high correlation ($r = 0.955$; $P<0.05$) between sensory overall score and sensory surface discoloration score. Results affirmed findings by Hood and Riordan (1973) who, as mentioned, reported linear increase in discrimination against discolored meat as metmyoglobin increased.

The relationship between sensory color scores and metmyoglobin concentration by absorbance also correlated ($r = -0.78$) with overall color score, $P<0.05$, and ($r = -0.76$) with surface discoloration score, $P<0.05$. These results indicated that the procedure described by Krzywicki (1979) assessing the relative content of myoglobin, metmyoglobin and oxymyoglobin is an acceptable method to determine the relative concentrations of myoglobin derivatives in meat.

Reflectance results from surface metmyoglobin concentration correlated poorly with sensory overall color score and surface discoloration score ($r = -0.23$, $P<0.05$, and -0.28, $P<0.05$). Reflectance results for surface metmyoglobin concentration also correlated poorly with absorbance ($r = 0.09$; $P<0.05$). Thus, surface metmyoglobin concentration by the reflectance measurement we used may not be a good indicator of surface discoloration for restructured steaks. One possible explanation for the low correlations may be the color variations in muscle chunks distributed in the restructured steaks. Hunt (1980) pointed out several causes for low correlations between visual and instrumental scores which included: discolor variation encountered in a meat surface causing a sensory panel to give "average" color scores, and spectrophotometers and colorimeters scanning a limited area of the meat surface that is not "average" for the entire meat surface. Although reflectance measurement is a nondestructive method to measure color on the surface of meat as observed by the consumer, the same system may be unsatisfactory for color measurements of restructured products which have wide variations in color.

CONCLUSION

BINDERS showed similar effects on initial color appearance of restructured steaks (except those containing calcium alginate or soy isolate protein) and over 12 wk frozen storage (except restructured steaks containing soy isolate protein). Calcium alginate, crude myosin extract, and wheat gluten could be used as effective binders in restructured beef steaks since they did not adversely affect product color or color stability.

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Assessment of the Textural Quality of Meat Patties: Correlation of Instrumental and Sensory Attributes

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ABSTRACT

Compression, shear, tensile and punch tests were used to assess the mechanical strength of a ground meat product and discriminate between effects of various treatments on this type of product. These measurements, together with chemical and moisture retention measurements, were related to sensory attributes of the cooked products. Most of the sensory textural attributes were satisfactorily (i.e., about 80% of the variance) explained or described by a combination of Warner-Bratzler shear parameters and certain compression measurements. Principal co-ordinate analysis showed that tensile measurements related better to sensory greasiness and juiciness than to mechanical strength. Methods for measuring juiciness were not adequate and indicated the need for further investigation.

INTRODUCTION

A WIDE VARIETY of mechanical methods have been developed and used for defining the texture of meat, and there has been some agreement that tenderness and juiciness adequately described sensory quality (Szczesniak and Torgeson, 1965; Harris and Shorthose, 1988). For manufactured meat products, which can cover a wide range of types (e.g., patties, restructured meat products, frankfurter and salami), there has been no general agreement on sensory attributes or on mechanical methods for any or all of the product types. It would thus be incumbent upon groups working on manufactured meat products to derive their own sensory profiles and to establish which, if any, mechanical methods relate to either the attributes in the profile or to the structure of particular products. Several workers have described the development and use of sensory profiles suitable for investigating the properties of ground meat products (Dransfield et al., 1984a. b; Berry and Leddy, 1984; Berry and Civille, 1986; Berry et al., 1987; Berry, 1987). A relatively new technique for deriving a sensory profile for a processed meat product (and then relating sensory scores to consumer ratings) has been described in a recent report (Jones et al., 1989). The use of this technique (free choice profiling) for ground meat products, both alone and preliminary to construction of a concensus profile (where the test panelists convert individual sensory profiles to a profile with an agreed set of attributes) has been described (Beilken et al., 1990, 1991a).

In much work reported on ground or processed meat products, there have been indications that the mechanical and structural properties of the products have a strong influence on both sensory attributes and consumer acceptability (Cross et al., 1978; Dransfield et al., 1984a, b; Bernal et al., 1988). It thus appeared that methods for measuring the physical/mechanical properties of manufactured meat products should be developed in parallel with sensory methods (Dransfield et al., 1984a, b; Bernal et al., 1988). Most such tests were performed on cooked products and cooking methods ranged from oven cooking (Randall and Larmond, 1977) to heating in a water bath (Gillet et al., 1978). Meat samples of fixed size were cooked in temperature controlled water baths (Harris and Shorthose, 1988) and similar cooking conditions could be used for ground meat products to ensure uniform and reproducible conditions.

In our study, the objectives were to investigate methods and techniques which would be suitable for measuring mechanical properties of ground meat products and then to compare the results, obtained using such methods, with sensory results obtained using a sensory profile.

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MATERIALS & METHODS

Material

Twelve types of ground meat patty products were used for these experiments and their compositions are listed in Table 1. The patties all weighed about 100g and were made using the same press so that initial dimensions and weights were nearly identical. The lean meat used for the various patties was obtained from 4 young (2–4 mo) and 3 old (2–4 yr) beef animals of unknown sex. The muscles used were obtained from the hindquarter cuts (i.e., topside, silverside, rump and knuckle) and included the semimembranosus, adductor, biceps femoris, gluteus medius, vastus lateralis and rectus femoris muscles. These muscles were removed from the carcasses at 24 hr post slaughter after chilling at 3–5°C, divided into convenient size pieces and batches suitable for grinding and flaking and then stored at −2.5°C until needed. Particle size was varied by flaking and by using different plate sizes (2, 5 or 12 mm) in the grinder. Samples were flaked using a Comitrol Flaker Model 3600 (Urschel Laboratories Inc., Valparaiso, IN) with a 2-J-030750 head for samples A and B and a 2-K-060510 head for K. The fat was from beef animals and was flaked frozen using a 2K-030-240-U head. Binding was increased (for samples B and D) by using a pressure treatment described elsewhere (Macfarlane et al., 1984). The pressure vessel was immersed in an ice-water mix-

Table 1 – Composition of various patties used in sensory trials

No.	Treatment[a]	Composition[bc] (%)
A	Flaked	Beef 78.7; Fat 20.0; NaCl 1.0; FL 0.3
B	Flaked + pressure	Beef 78.7; Fat 20.0; NaCl 1.0; FL 0.3
C	Ground	Veal fine 75.3, medium 22.7; NaCl 1.0; TSPP 0.5; FL 0.05
D	Ground + pressure	Veal fine 40.0, medium 38.0; Fat 20.0; NaCl 1.0: TSPP 0.5; FL 0.5
E	Ground (Commercial Product)	Beef 997; FL 0.3
F	Ground	Beef coarse 88.2; NaCl 1.0; TSPP 0.5; H_2O 10.0; FL 0.3
G	Ground	Beef coarse 80.0; Fat 10.0; NaCl 1.0; H_2O 5.7; BR 3.0; FL 0.3
H	Ground	Beef coarse 36, medium 36; Fat 10.0; NaCl 1.0; H_2O 10.0; BR + WH 4.5; CL + P 2.0; FL 0.5
I	Ground	Beef medium 61.7; Fat 9.0; NaCl 1.0; TSPP 0.5; H_2O 8.0; BR + WH 13.0; CI + P 6.5; FL 0.3
J	Ground	Beef fine 44.0; Fat 10.5; NaCl 1.0: TSPP 0.5; H_2O 25.0; BR + WH 15.6; CL + P 3.0; FL 0.4
K	Flaked	Beef 60.0; Fat 20.0; NaCl 1.0; TSPP 0.3; H_2O 14.0; BR + WH 3.8; P 0.4; FL 0.5
L	Ground	Beef fine 40.0; Fat 23.0; NaCl 1.0; H_2O 22.0; BR 7.6; Flour 4.4; CL 1.6; FL 0.4

[a] Plate sizes for grinding: fine 2 mm, medium 5 mm, coarse 12 mm.

[b] BR – breadcrumbs WH – wheat germ; TSPP – tetrasodium pyrophosphate; CL – Lindgren Promine DS soy protein concentrate; P – Lindgren Procon 2060 granulated soya (similar to textured vegetable protein).

[c] All formulations included small amounts of flavoring material (FL).

Table 2—Consensus profiie derived for patties listing the attributes and an indication of their interpretation or meaning

No.[a]	Sensory attribute[b]	Interpretation[c]
1.	Rubberiness (apparent)	Press with the flat of a fork.
2.	Adhesion (apparent)	Cut with the side of a fork.
3.	Rubberiness	Initial sensation on biting and chewing.
4.	Crumbliness	Nature and ease of initial breakdown on chewing.
5.	Coarseness	Nature and size of particles making up the mix.
6.	Chewiness of particles	Nature and breakdown by chewing of particles in mix.
7.	Juiciness	Sensation of wetness and juiciness.
8.	Greasiness	Sensation of fattiness in mouth.
9.	Overall texture	Acceptability of texture of sample.

[a] For 1-6 low – high
7 dry – juicy
8 nil – high
9 very poor – very good

[b] Attributes 1 and 2 were determined by pressing or cutting with a fork before mastication.

[c] All measured by marking on a 100 mm line with the ends labelled low (0) to high (100) except for juiciness which was dry (0) to juicy (100), overall texture which was very poor to very good (100) and greasiness nil (0) to high (100).

ture during pressure treatment of the frozen patties (150 MPa for 20 min).

The fat, water, cereal and soy protein contents were altered to affect texture and juiciness. Several formulations were similar in texture to commercially available samples but only sample (E) was obtained from a commercial source and was, nominally, 100% beef. A small amount of flavoring (0.3–0.5%) was added to each formulation to minimize flavor differences which, in earlier experiments using commercial samples, had been appreciable (Beilken et al., 1991b). Moisture, fat and protein contents of raw and cooked samples of each of the types of patties were measured using standard AOAC (1975) methods.

Cooking methods

Samples for the sensory panel were cooked from the frozen state on a commercial device which consisted of two temperature-controlled hot plates mounted one above the other. The plates were separated by a distance sufficient to allow samples to change shape during cooking without being restricted by the hot plates. The plates were covered in aluminum foil and temperature settings were adjusted so that the standard 100-g patties heated for 12 min (turned after 6 min to reduce local burning) reached an internal temperature of 80 ± 1°C. After cooking, each patty was cut diametrically into four approximately equal parts which were placed directly into polyethylene bags and into polystyrene cups. These cups were then covered with aluminum foil and placed in an incubator at 50 ± 2°C for up to 10–15 min until needed. Using this method, samples were at 55–60°C when served to the taste panel.

The samples for mechanical assessments were cooked by placing each 100-g sample in a polyethylene bag, fastening with a metal clip and totally immersing in a water bath maintained at 80 ± 0.5°C for 60 min. After cooking, the samples were cooled in running water (23 ± 2°C) for at least 30 min. This cooking method was chosen as it was convenient for handling large numbers of samples, was easy to reproduce, yielded uniformly cooked samples, and provided samples suitable for cutting into the sub-samples for mechanical assessment.

Measurement of dimensional changes, moisture loss and pH

All the patties were made in the same mold/press so that initial areas and weights were similar (about 100g). The cross-sectional areas, both before and after cooking, were measured using a grid normally used for measuring muscle areas on beef carcasses. Patties from each formulation were weighed (before and after cooking) first in air and then in a liquid of known density. Volume changes on cooking were then calculated. The pH was measured on the raw samples at room temperature (23 ± 2°C) using a Philips C64/1 combined electrode with a Townson expanded scale meter. Expressible juice was measured using a centrifugal method previously employed for meat (Bouton et al., 1972a,b). Samples weighing about 4g were centrifuged at 100,000g for 1 hr in stainless steel tubes with aluminum caps using a 50 Ti rotor in a Beckman Spinco Model L centrifuge.

Mechanical measurements

Punch tests. A whole patty was placed over a 30 mm i.d. hole and a plunger (o.d. 28 mm), centrally positioned over the hole, was driven completely through it. The plate with the hole in it had been mounted on an Instron 1122 with a 0-500 kg load cell so the force required to punch the hole through the patty could be measured. This measurement was carried out on both raw and cooked (80°C water bath for 1 h) patties. The crosshead speed was 100 mm/min.

Tensile test. The device previously used on ground meat products (Macfarlane et al., 1984) was used. Samples 2.5 cm wide, 1 cm thick and 4–5 cm long were cut from each patty. Tensile force required to rupture the samples was measured using an Instron 1122 set up in its tensile mode, with crosshead speed of 50 mm/min.

Instron compression. This method has been reported previously for determining meat tenderness (Bouton and Harris, 1972). The force and work required to push a plunger (o.d. 6.3 mm) 80% of the way through a 1 cm thick sample and then the work done on a second penetration of the plunger in the same location was used to measure: hardness—the height of the first peak; cohesiveness—ratio of work done on second penetration to that done on the first; and hardness x cohesiveness (the Instron Compression or IC measurement, i.e., chewiness).

Warner-Bratzler shear. For this version of the Warner-Bratzler (WB) shear device, samples were cut into sub-samples with 1.5 × 1.5 cm square cross sections and 4–5 cm long. The shear blade was 3.41 mm thick with gap width 0.63 mm. Initial yield force, slope at initial yield and peak force were measured from force deformation curves. At least 6 shear measurements were carried out on each patty sample.

Compression III measurement. Samples were cut into smaller samples with a rectangular cross section (1.5 × 1.0 cm) and 2.5 cm long. These were compressed 80% (i.e., from 1.5 to 0.3 cm) beneath a 6.5 cm o.d. plunger. Initial yield force, initial yield distance, slope at initial yield and peak force were measured from the force-deformation curves.

Sensory methods

The derivation of the consensus sensory profile has been described in detail (Beilken et al., 1991a). In brief, the profile was compiled using twelve panellists, all very experienced in meat texture work, who were instructed to ignore flavor and consider textural attributes of patties. The profile they produced, plus an interpretation of what each attribute meant to them, is shown in Table 2. The samples were presented warm, one at a time, on a white paper plate (with a three digit code) under red lighting in individual booths. The arrangement of samples at each session was according to a balanced incomplete block design for 12 samples (Design number SR 22, Bose et al., 1954). According to this design, each sample was tasted four times in eight sessions at each of which six samples were presented.

Statistics

Statistical analyses were carried out using the GENSTAT computer package (Genstat 5, copyright 1987, Lawes Agricultural Trust, Rothamsted Experimental Station). Analysis of variance was used to ascertain treatment effects and, where appropriate, standard errors and hence least significant differences (LSD) were computed. A principal co-ordinate analysis also was performed, using standardized data, to assess the variation of the products over the mechanical/chemical measurements. The principal co-ordinate analysis was performed instead of a principal components analysis because the number of variables exceeded the number of data units. Principal components analysis was carried out on the correlation matrix of the attribute means for the consensus sensory profile. Forward stepwise multiple regression procedures were used to obtain estimation equations for sensory components in terms of mechanical measurements.

RESULTS & DISCUSSION

Composition of experimental patties

The formulations and treatments used for the experimental patties have been listed in Table 1 with the water, fat and protein contents of the raw patties listed in Table 3. The water, fat and protein contents of the patties cooked by both methods were also measured but because they were similar to the raw

Table 3—Some physical and compositional properties of 12 types of meat patties

Measurement[a]	Patty identification[b] A	B	C	D	E	F	G	H	I	J	K	L	LSD[c]
% cooking loss	30.4	28.5	9.1	7.8	32.8	25.4	25.8	20.2	0.7	0.5	8.2	4.8	2.4
% change in CS area	37.1	38.7	22.4	26.5	43.7	30.7	34.0	33.9	13.3	10.3	24.0	21.5	3.2
pH	5.59	5.71	6.06	6.20	5.96	5.83	5.57	5.69	6.08	6.18	5.95	5.65	0.06
% EJ - raw	11.0	13.0	5.5	0.1	0.5	16.7	26.6	25.0	0.1	0.1	0.7	22.8	2.3
% EJ - cooked	3.5	2.3	6.2	7.4	7.2	21.7	9.1	9.3	1.4	6.6	6.4	2.6	2.9
% Moisture[d]	60.0	60.9	75.3	60.6	58.1	75.8	67.3	66.2	57.4	62.9	60.2	56.3	–
% Fat[d]	20.4	20.3	1.7	21.0	25.4	2.6	1.09	11.6	9.0	11.1	21.5	22.2	–
% Protein[d]	17.4	15.9	20.3	16.5	15.6	18.7	17.9	16.7	18.9	12.2	13.8	11.6	–

[a]CS = cross section, EJ = expressed juice
[b]Patty identification as in Table 1
[c]Least significant differences at P < 0.05
[d]LSD's not computed for these values which were means of two replicates within 5%

Table 4—Some mechanically measured physical properties of 12 types of meat patties

Mechanical method[a]	Patty identification No.[b] A	B	C	D	E	F	G	H	I	J	K	L	LSD[c]
Punch–raw	4.30	3.78	0.79	1.40	2.14	1.43	2.05	1.04	2.25	0.59	0.79	0.58	0.32
Punch–cooked	8.90	10.44	8.22	8.45	6.83	8.48	7.60	6.64	14.02	6.37	9.11	3.79	0.93
Tensile	0.67	0.85	1.26	1.16	0.42	1.18	0.69	0.79	1.90	0.96	1.01	0.49	0.12
Instron compression													
Hardness (H)	2.65	2.73	1.11	1.25	1.45	2.30	1.72	1.42	2.08	0.93	1.16	0.61	0.22
Cohesiveness (Coh.)	0.24	0.22	0.19	0.16	0.25	0.27	0.24	0.18	0.15	0.14	0.19	0.13	0.02
H × Coh.	0.62	0.62	0.22	0.21	0.36	0.63	0.40	0.26	0.31	0.13	0.22	0.08	0.08
WB shear													
Initial yield force–IY	2.95	3.08	2.11	2.10	1.43	2.54	1.41	1.46	3.45	1.61	1.67	0.75	0.33
Slope at yield	3.1	2.8	4.1	4.0	4.5	3.9	4.2	3.7	2.4	4.6	4.3	7.0	0.7
Peak force–PF	4.96	4.92	2.27	2.34	2.87	3.45	2.57	2.35	4.00	1.83	2.42	0.99	0.45
Compression III													
Initial yield force–IY	2.0	3.0	4.0	5.3	2.3	3.1	1.7	1.5	1.5	2.8	2.3	1.0	0.9
Initial yield distance–IYD (%)	14.7	24.0	40.0	50.7	28.0	36.0	17.3	17.3	10.7	42.7	32.0	21.3	6.9
Slope at yield	8.8	9.6	8.6	9.4	5.9	6.3	6.7	5.7	11.9	4.4	5.1	3.3	1.2
Peak force–PF	47.2	43.0	21.6	20.6	27.0	30.1	33.4	22.6	24.8	12.0	18.7	9.1	4.8

[a] All in kg except for cohesiveness (a ratio), WB slope at yield (kg/cm) and Compression III IYD (%) and slope at yield (kg/cm)
[b] See Table 1 for patty identification
[c] Least significant difference at p < 0.05

data, they have not been listed. Many of the formulations also contained carbohydrate and other materials. Sample E, a commercial product, was, nominally, 100% beef and had high fat content.

Mechanical measurements

The cooking losses, cross sectional area changes, pH and centrifugally expressed juice (from both raw and cooked) results obtained for all 12 patty types are listed in Table 3. As expected, the samples containing high proportions of meat lost the most weight during cooking, whereas those containing high amounts of cereal lost least. Exceptions were the two samples (C and D) containing large amounts of veal which had lower losses than expected. There was no obvious explanation except that the easily gelatinized collagen in veal may have improved moisture retention. The decreases in cross sectional area (Table 3) showed that by increasing cereal content (e.g., I and J) samples were produced where size did not change much during cooking. The range in pH values was not large (i.e., 5.57–6.20) and did not appear to have much effect on expressed juice values from raw or cooked samples. Expressible juice for cooked samples was, with the exception of F, quite low. Such lack of free moisture would, in table meat, have indicated dry (i.e., nonjuicy) samples.

The mean results for all mechanical type measurements are listed in Table 4. All of the measurements had highly significant differences between most samples and, hence, between treatments. The only raw to cooked comparison was for the punch tests where cooking increased resistance to penetration. The veal samples (C and D) compressed over 40% before yield (Compression III) and had the highest initial yield values (Compression III).

Table 5—Principal co-ordinate analysis of standardized data for mechanical/chemical measurements

Measurement no.	Measurement[a]	Principal co-ordinate loadings 1	2	3	4
1	Punch–raw	28	8	−6	0
2	Punch–cooked	25	−13	−1	3
3	Tensile peak force	10	−21	4	3
4	WB Initial yield force	28	−10	0	0
5	WB Slope at yield	−27	8	−3	0
6	WB Peak force	31	2	−2	0
7	IC Cohesiveness	17	15	7	4
8	IC Hardness	30	4	1	0
9	IC Chewiness	27	9	4	−2
10	Comp. III Initial yield force	3	−11	2	−13
11	Comp. III Initial yield distance	−12	−9	2	−12
12	Comp. III Peak force	29	9	1	−2
13	Comp. III Slope at yield	25	−12	−1	0
14	% Cooking loss	16	18	2	−5
15	pH raw	−10	−20	3	−2
16	% Centrifugally exp. juice–raw	−5	20	8	2
17	% Centrifugally exp. juice–cooked	−15	8	14	2
18	% Moisture	−2	1	18	−2
19	% Fat	0	8	−17	−4
20	% Protein	−18	−11	10	2

[a] Warner-Bratzler = WB; IC = Instron compression and Comp. III = Compression III measurements

To reduce the complexity of comparing the 12 products over the 20 instrumental measurements (Table 5), a principal co-ordinates analysis was performed and the first four components of the analysis considered. The total percentage variation explained by using four dimensions or components was 91.5%. The first component (Table 5) accounted for 42.2% and related mainly to Instron Compression (IC), compression III, punch and WB shear measurements. The second component accounted for 24.7% and reflected mainly tensile force and pH

Table 6—Means of consensus sensory profile attributes (N=48) measured for the 12 varieties of patty

Sensory attribute[a,b]	Patty Identification No.[c]												
	A	B	C	D	E	F	G	H	I	J	K	L	LSD[d]
Rubberiness (App)	62.9	72.3	67.8	61.9	58.7	73.6	65.3	58.4	79.6	63.3	53.9	25.3	6.0
Adhesion (App)	72.4	76.6	61.4	44.1	59.3	76.7	52.5	46.2	75.8	46.3	44.7	16.4	6.9
Rubberiness	63.2	65.0	53.3	42.2	52.3	64.8	45.7	35.2	53.8	34.7	34.4	10.6	5.5
Crumbliness	37.6	28.7	52.8	46.7	57.6	36.9	46.3	53.2	46.7	51.6	52.0	58.6	8.6
Coarseness	72.5	71.1	32.7	26.4	61.6	65.3	67.4	44.3	36.5	24.4	41.7	16.3	5.7
Chewiness of particles	72.2	73.5	34.1	26.7	63.0	69.7	62.1	37.0	39.3	20.1	37.4	11.0	5.9
Juiciness	58.5	55.8	36.4	53.2	33.3	42.2	46.2	47.6	32.0	38.9	56.5	40.8	11.4
Greasiness	52.9	55.4	41.2	55.8	45.9	33.0	46.7	52.2	42.4	54.0	54.0	62.9	9.1
Overall texture	51.0	51.8	33.3	29.4	48.8	46.5	57.7	46.9	32.2	29.4	49.4	14.4	8.0

[a] App = apparent
[b] All attributes were scored on a scale of 0-100
[c] See Table 1 for patty composition and treatment
[d] Least significant difference at P<0.05

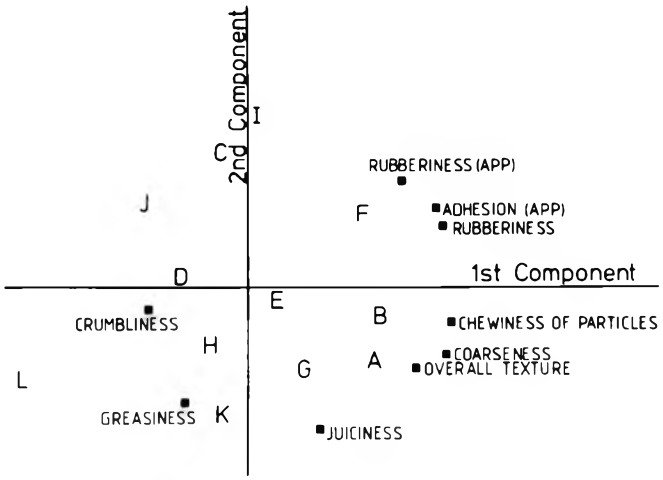

Fig. 1—Plot of the 12 patty types (A—L) relative to the first two principal components of the consensus sensory profile data. The attributes of the sensory profile have also been plotted using as co-ordinates the correlation coefficients relative to each axis.

contrasting with moisture measurements (expressed juice and cooking loss). The third component (15.3%) related mostly to percent moisture and percent expressed juice (cooked samples) contrasting with percent fat. The fourth component (9.4%) related mainly to compression III measurements of initial yield force and initial yield distance.

Sensory results

Results for the consensus profile, i.e., eight sensory attributes and one rating (overall texture) have been listed in Table 6—for all 12 types of patties. Principal components analysis of the sensory data was carried out and positions of the various patties were plotted relative to the first and second principal axes (Fig. 1). Also plotted in Fig. 1 are the locations of the sensory attributes relative to the principal component axes. The co-ordinates of each attribute were obtained using its correlations relative to the two axes. Each letter (A to L) in Fig. 1 represented the mean of four replications for each of 12 patties. From Fig. 1, samples A and B were coarser and had chewier particles than L, C and J. The samples in the lower quadrants were considered juicier and greasier than those in the upper quadrants. Sensory results have been discussed in greater detail elsewhere (Beilken et al., 1991a). The main emphasis of this report is on mechanical measurements and the relationship between those measurements and sensory results.

Relationships between mechanical and sensory measurements

Correlations between mechanical measurements and consensus profile sensory measurements are listed in Table 7. The raw punch measurements related best to crumbliness, coarseness and chewiness of particles. Punch tests on the cooked samples related best with the attributes assessed before mastication viz rubberiness and adhesion. Tensile measurements related ($P<0.05$) only to rubberiness assessed before mastication. With compression III measurements, the slope at initial yield values had highly significant relationships with rubberiness, coarseness and chewiness of particles. For the WB shear device, "slope at initial yield," initial yield force and peak force all correlated highly with rubberiness and adhesion (both assessed prior to mastication). Peak force values related best to rubberiness and crumbliness and only adhesion (appear-

Table 7—Correlation coefficients between mechanical measurements and the nine consensus profile sensory attributes

Mechanical measurement	Sensory attributes[ab]								
	1	2	3	4	5	6	7	8	9
Punch—raw	0.39	0.67*	0.70*	−0.73**	0.76**	0.78**	0.41	−0.01	0.54
Punch—cooked	0.76**	0.76**	0.64**	−0.53	0.28	0.37	0.04	−0.41	0.27
Tensile	0.62*	0.43	0.29	−0.20	−0.26	−0.16	−0.26	−0.47	−0.22
Instron compression									
Hardness (H)	0.66*	0.86***	0.86***	−0.89***	0.83***	0.87***	0.34	−0.35	0.63*
Cohesiveness (Coh.)	0.42	0.61*	0.72**	−0.48	0.91***	0.92***	0.19	−0.55	0.82***
H × Coh.	0.57	0.81***	0.85***	−0.85***	0.92***	0.95***	0.35	−0.42	0.70*
WB shear									
Initial yield force—IY	0.77**	0.87***	0.80**	−0.76**	0.40	0.50	0.15	−0.38	0.23
Slope at yield	−0.88***	−0.86***	−0.80**	0.65*	−0.52	−0.57	−0.20	0.42	−0.54
Peak force—PF	0.65*	0.88***	0.86***	−0.83***	0.75**	0.81**	0.34	−0.27	0.57
Compression III									
Initial yield—IY	0.35	0.17	0.31	−0.23	−0.13	−0.04	0.17	−0.15	−0.10
IY distance	0.04	−0.19	−0.07	0.11	−0.39	−0.33	0.01	−0.04	−0.31
Slope at IY	0.53	0.76**	0.82***	−0.79**	0.91***	0.92***	0.46	−0.24	0.74**
Peak force—PF	0.70*	0.70*	0.67*	−0.54	0.25	0.34	0.06	−0.31	0.14

[a] 1—Rubberiness (apparent), 2—Adhesion (apparent), 3—Rubberiness, 4—Crumbliness, 5—Coarseness, 6—Chewiness of particles, 7—Juiciness, 8—Greasiness, 9—Overall texture
[b] * P<0.05; ** P<0.01; *** P<0.001

Table 8—Regression equations relating the various sensory attributes with mechanical measurements

Sensory attribute	Regression equation[a]	% variance explained[b]
Rubberiness—apparent	y = 11.0 − 10.07 WB slope − 0.52 % Fat	74.9, 81.5
Adhesion—apparent	y = 27.9 + 13.63 WBPF − 0.84 % Fat	74.2, 87.5
Rubberiness	y = −10.2 + 9.43 WBPF + 2.06 % Prot.	70.6, 79.6
Crumbliness	y = 15.4 − 13.41 Hard. − 0.23 Compression III IYD	76.9, 84.3
Coarseness	y = −12.9 + 53.0 Chew. + 211.7 Coh.	82.3, 89.9
Chewiness of particles	y = −15.7 + 67.4 Chew. + 195.4 Coh.	89.1, 94.8
Juiciness	y = 58.2 + 0.52 Compression III PF − 1.9 % Prot.	12.9, 36.5
Greasiness	y = 87.4 − 3.59 % Prot. + 1.78 Compression III PF	59.8, 73.2
Overall texture	y = 15.9 + 196.6 Coh. − 3.37 WB slope	63.6, 69.5

[a] WB = Warner Bratzler; IY = Initial yield force; PF = Peak force; IYD = Initial yield distance; Hard = Instron Compression peak; Chew = Hard × cohesiveness; Coh. = IC cohesiveness

[b] For best single and double variable prediction equations

ance). Instron compression value correlated highly with several attributes but primarily coarseness and chewiness of particles.

There are many ways of determining how well the mechanical measurements describe the sensory data. One way is to use forward stepwise multiple regression analysis which starts from the best single predictor and determines the benefit of adding further predictors, one at a time. The results are shown in Table 8. With exception of juiciness (36.5% variance explained), greasiness (73.2%) and overall texture (69.5%), most of the attributes required only two measurements (either mechanical or chemical) to explain near or beyond 80% of the variance. The juiciness results were extremely low, indicating none of the measurements truly reflected this attribute.

Another way to assess relationships of mechanical measurements to sensory data was to construct a correlation plot. For each mechanical measurement, the correlation with the first and second principal component axes of the sensory analysis were used to provide a plot of these measurements (Fig. 2). The first component was mainly associated with measurements of mechanical strength while the second component was mainly associated with compositional differences.

Results in Fig. 2 can be compared directly with those in Fig. 1. The Instron Compression measurements related to the attributes of rubberiness, chewiness and coarseness as did the WB peak force and compression III peak force. This reflected the fact that the first component related to mechanical strength. The tensile measurements surprisingly appeared to relate better to greasiness and juiciness than to any measurement, sensory or otherwise, of mechanical strength.

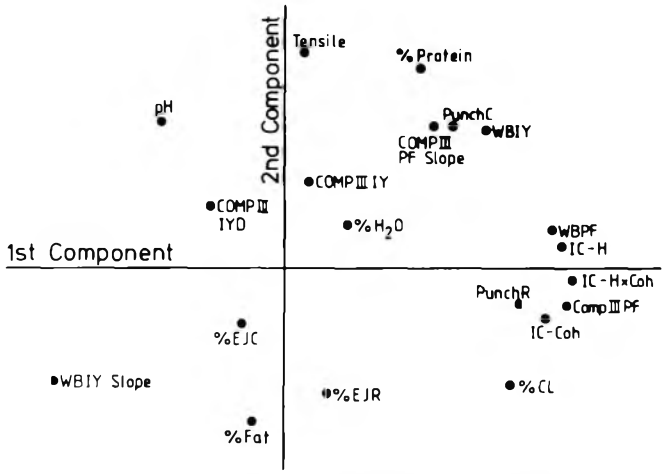

Fig. 2—Plot of the objective measurement correlations relative to the first two principal components of the consensus profile data. WB—Warner Bratzler; Comp III—Compression III; IY—initial yield force; IYD—initial yield distance; PF—peak force; IC—Instron compression; H—hardness; Coh—cohesiveness; EJ—expressed juice; CL—cooking loss; R—raw; and C—cooked.

Implications of results

Some of the mechanical measurements distinguished between treatments and/or types of patty likely to be encountered in commerical situations. Direct comparisons of this work with similar reported work presented some difficulty. There have been very few reports of mechanical methods compared with attributes of a sensory profile. In the UK, a punch and die method was used on raw and grilled patties (Jones et al., 1985) and found to correlate well with some sensory attributes. In Canada, compression, WB shear and tensile methods were related to sensory assessments (Bernal et al., 1988) and the WB shear measurements correlated highly with several sensory measurements. For ham samples shear strength, tensile strength and stress relaxation were measured (Nute et al., 1987) and 'gave a good indication of the texture'.

Another objective of our work was to determine whether mechanical measurements on ground meat products cooked in a reproducible, controlled way (80 ± 0.5°C for 60 min in a water bath) could be compared with sensory results on similar samples conventionally cooked (internal temperature of 80 ± 1°C). Apart from methods for measuring juiciness (which were clearly inadequate and needed to be improved), the mechanical measurements related well to sensory data. Similar results on sausages were obtained for mechanical measurements from samples cooked in a water bath at 80 ± 0.5°C and those cooked conventionally. Further work may show whether mechanical properties of the patties were similar for the two cooking methods or whether the mechanical measurements reflected structural strength which was sensorily important.

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Assessment of the Sensory Characteristics of Meat Patties

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ABSTRACT

The use of two different methods for determining sensory profiles for texture of meat patties was investigated and compared with consumer ratings. The individual sensory profiles developed in free-choice profiling were analyzed using generalized Procrustes and principal components analysis techniques and converted by discussion to a single consensus profile. Using three components of the principal components analysis the free-choice profile accounted for 88.2% of the total variance in texture and the consensus profile for more than 90%. The first components related mostly to mechanical strength attributes and the second to greasiness and juiciness. Both sensory profiles were shown, using preference mapping techniques, to be good indicators of consumer acceptability.

INTRODUCTION

THE SENSORY and mechanical methods developed at this and other laboratories for defining and quantifying the quality attributes of meat per se appear to be adequate (Harris and Shorthose, 1988). A study of the literature indicated that this was not the case for manufactured meat products such as patties, restructured meats and sausages of various types (including semi-fermented sausages such as salami) probably due to the greater complexity of their texture. Sensory profiles developed for these product types have contained up to 19 textural attributes (Berry and Leddy, 1984; Berry and Civille, 1986; Berry et al., 1987). Less complicated profiles have, however, been reported (Dransfield et al., 1984) which contain only six attributes, i.e., rubberiness, ease of fragmentation, degree of comminution, character of the particles, moistness and overall texture.

There are many ways in which sensory profiles can be developed (Dransfield et al., 1984; Berry and Civille, 1986; Jones et al., 1989). It has, however, been claimed (Williams and Langron, 1983; Williams and Arnold, 1985; Jones et al., 1989) that free-choice profiling (FCP), a sensory testing technique, has advantages. It reduces time spent in training the panel and enables testing of the reliability and equivalence of a large number of descriptors of different attributes of a particular product type. In FCP the assessors develop their own individual list of terms for describing similarities and differences among samples of a particular product type. The collection of scores for the samples from each assessor forms an assessor "sample space" and a mathematical technique (generalized Procrustes analysis or GPA - Gower, 1975) is then used to combine scores. In GPA these "sample spaces" are transformed by translation, rotation and scaling. Results from individual panelists, using various descriptors and narrow or wide ranges of scores when comparing samples, can be rationalized. The GPA thus allows individual variation in terms or descriptors of the panelists to be examined by correlating them with principal axes of the centroid of the assessor sample spaces (Jones et al., 1989). When developing FCP for a particular product type, it is desirable to provide a range of the product type (i.e., different textural or other properties) and a sufficient number of different samples to enable significant correlations to be obtained.

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The prime concern of a sensory panel is to assess and quantify the sensory characteristics of different products or product types while acceptability is probably best determined by consumers. There are a variety of ways consumer tests can be carried out, e.g., home tests where families test a limited number of products at a time or hall tests where up to 10–12 samples can be presented in conditions similar to those in the laboratory (Jones et al., 1989).

From the data collected from a trial of the hall type, it is possible to relate consumer preferences to laboratory panel data. A model that allows different preference patterns among consumers can be formulated and this overcomes the possibility of opposing views cancelling each other when simple averages of preference data are calculated. A procedure, known as preference mapping (MacFie and Thomson, 1983) uses linear or quadratic regression to express the scores of each consumer in terms of sensory dimensions defined by a laboratory panel. There is thus a gain in ability to segment consumer populations by presenting them with a range of products as opposed to giving a large number of consumers a subset of products as in home tests.

In our study the use of FCP has been investigated (both alone and as a precursor to setting up a more conventional fixed choice or consensus profile - where the panelists agree on appropriate attributes) for determining the relevant sensory attributes of a ground meat product. In addition we investigated the use of preference mapping (Jones et al., 1989; MacFie and Thomson, 1983) in relating laboratory sensory data to the performance of consumers.

MATERIALS & METHODS

Materials

For the FCP, the consensus profile and the consumer survey patties that covered a range of textures were made at the laboratory. In making them clearly some ingredients imparted a characteristic flavor so a similar amount of flavoring was added to each to reduce such flavor differences. The composition of the patties, where meat percentage is for lean meat (including residual fat), is listed in Table 1. All patties weighed about 100g and were made in the same press so that initial dimensions and weights were similar. After manufacture, patties were stored at −25°C until needed.

The meat used for the patties was obtained from the hindquarter cuts of 4 young (2–4 months) and 3 older (2–4 year) beef animals of unknown sex. These hindquarter cuts included the topside, silverside, rump and knuckle and the muscles involved were the semimembranosus, adductor, biceps femoris, semitendinosus, gluteus medius, vastus lateralis and rectus femoris. Muscles were removed from the carcass after 24 hr chilling at 3–5°C, trimmed of all obvious fat and connective tissue and then divided into convenient size pieces suitable for grinding and flaking. These meat pieces were further divided into 2–3 kg packages which were frozen and stored at −25°C up to 2 wks. When required packages were conditioned at −2.5°C before further processing.

Particle size was varied by flaking and by using different plate sizes (2, 5 or 12 mm) in the grinder. Samples required for A, B and K were flaked using a Comitrol Flaker Model 3600 (Urschel Laboratories Inc., Valparaiso, IN) equipped with a 2-J-030750 head for A and B and a 2-K-060510 for K. The fat was from beef animals and was flaked from the frozen state using a 2-K-030-240-U head.

Pressure treatment used for Samples B and D has been described previously (Macfarlane et al., 1984) and was used to increase binding and hence rubberiness. The pressure vessel was immersed in an ice-water mixture during treatment and the patties were frozen before pressure treatment (150 MPa for 20 min). Texture was also varied by changing the fat, water, cereal and soy protein contents.

Table 1—Formulation of meat patties with different textures used in sensory trials

Variation	Treatment[a]	Meat	Percentage composition[b]			
			Fat	NaCl	H_2O	Other
A	Flaked beef	78.7	20.0	1.0	–	FL 0.3
B	Flaked beef + pressure	78.7	20.0	1.0	–	FL 0.3
C	Ground veal	Fine 75.3 Medium 22.7	–	1.0	–	FL 0.5; TSPP 0.5
D	Ground veal + pressure	Fine 40.0 Medium 38.0	20.0	1.0	–	FL 0.5; TSPP 0.5
E	Ground beef – commercial	99.7	–	–	–	FL 0.3
F	Ground beef	Coarse 88.2	–	1.0	10.0	FL 0.3; TSPP 0.5
G	Ground beef	Coarse 80.0	10.0	1.0	5.7	FL 0.3; BR 3.0
H	Ground beef	Coarse 36.0 Medium 36.0	10.0	1.0	10.0	FL 0.5; BR + WH 4.5; CP + P 2.0
I	Ground beef	Medium 61.7	9.0	1.0	8.0	FL 0.3; TSPP 0.5; BR + WH 13.0; CP + P 6.5
J	Ground beef	Fine 44.0	10.5	1.0	25.0	FL 0.4; TSPP 0.5 BR + WH 15.6 CP + P 3.0
K	Flaked beef	60.0	20.0	1.0	14.0	FL 0.5; TSPP 0.3; BR + WH 3.8; P 0.4
L	Ground beef	Fine 40.0	23.0	1.0	22.0	FL 0.4; BR 7.6; Flour 4.4; CP 1.6

[a] Plate sizes for grinding: fine 2 mm, medium 5 mm, coarse 12 mm

[b] BR—breadcrumbs; WH—wheat germ; TSPP—tetrasodium pyrophosphate; C—Lindgren Promine DS soy protein concentrate; P—Lindgren Procon 2060 granulated soya (similar to textured vegetable protein); FL—Flavoring material made up from monosodium glutamate, sugar, herbs and spices.

Table 2—Consensus profile derived for patties listing the attributes and an indication of their interpretation or meaning

No.	Sensory attribute[a]	Interpretation[b]
1	Rubberiness (apparent)	Press with the flat of a fork.
2	Adhesion (apparent)	Cut with the side of a fork.
3	Rubberiness	Initial sensation on biting and chewing.
4	Crumbliness	Nature and ease of initial breakdown on chewing.
5	Coarseness	Nature and size of particles making up the mix.
6	Chewiness of particles	Nature and breakdown by chewing of particles in mix.
7	Juiciness	Sensation of wetness and juiciness.
8	Greasiness	Sensation of fattiness in mouth, i.e., mouth coating.
9	Overall texture	Acceptability of texture of sample

For 1–6 low–high; 7 dry–juicy; 8 nil–high; 9 very poor–very good.

[a] Attributes 1 and 2 were determined by pressing or cutting with a fork.

[b] All measured by marking on a 100 mm line with the ends labelled low (0) to high (100) except for juiciness which was dry (0) to juicy (100), overall texture which was very poor (0) to very good (100) and greasiness nil (0) to high (100).

Cooking methods

The frozen samples for the laboratory taste panel were cooked on a commercial machine (Cook n' Grill, Breville Holdings Pty Ltd., Sydney, Australia). This device comprised two temperature-controlled hot plates mounted one above the other so that samples were cooked between them. The plate separation was adjusted so the upper plate was not in contact with the samples so that shape changes during cooking could proceed unaffected. The plates were covered in aluminum foil and temperature settings adjusted so that standard 100g patties, heated for 12 min (turned after 6 min to reduce local burning), reached an internal temperature of 80 ± 1°C. After cooking, each patty was cut diametrically into four approximately equal parts. These samples were placed directly into polyethylene bags in polystyrene cups which were covered with aluminum foil before placing in an incubator at 50 ± 2°C until needed (usually 10–15 min after cooking). Using this method, the sample temperatures at the time of serving to the taste panel were about 55–60°C. All temperatures were measured using iron-constantan thermocouples inserted near the geometric centers of the patties.

Samples for the consumer trial had to be cooked at the laboratory (using the same standard cooking and storage conditions used for laboratory sensory samples) since storage and cooking facilities were not available at the shopping center. After cooking, when samples were in polystyrene cups and had been covered with aluminum foil, they were placed inside a large polystyrene container for transporting to the shopping center. While the center was not far from the laboratory, the time delay between cooking and serving was unavoidably 4–6 min longer than at the laboratory.

Cooking conditions for the laboratory and consumer samples were as nearly identical as possible. However, unlike the laboratory taste panel, there were occasional delays in getting consumers into the test area and a microwave oven was then used to ensure that sample temperatures were initially 55–65°C.

Sensory methods

Twelve panelists, very experienced in meat texture work but relatively inexperienced in manufactured meat products, took part in both the FCP and in the consensus profiling. For the FCP they were asked, after tasting samples of different types of patty, to write down independently what they felt was a vocabulary or list of attributes adequately describing textural attributes of the patties. They were asked to ignore flavor and assess texture. No limit was placed on the number of attributes, and others could be added at any time. Each attribute was scored on a 100 mm line scale with labels for the ends of the line also selected by each panelist. At further trial sessions each panelist tasted several samples and finalized their list of attributes, i.e., established their individual profiles. Both at these trials and in the true panel sessions samples were presented warm, one at a time, on a white paper plate (with a three-digit code marked on the plate) under red lighting in individual booths. The arrangement of samples at each session was according to a balanced incomplete block design for 12 samples (the design used was SR22 in Bose et al., 1954). According to this design, each sample was tasted 4 times in 8 sessions at which 6 samples were presented. Each panelist was provided with water (if required to rinse between tastes) and most ate all the samples although suitable receptacles were provided if they did not.

After the FCP panels were completed, each panelist went through their individual profiles and discussed with other panelists the meaning of each attribute in terms of structure of the patty. From these discussions a consensus profile was produced. Once the panelists were satisfied that the new profile satisfactorily described the patty samples,

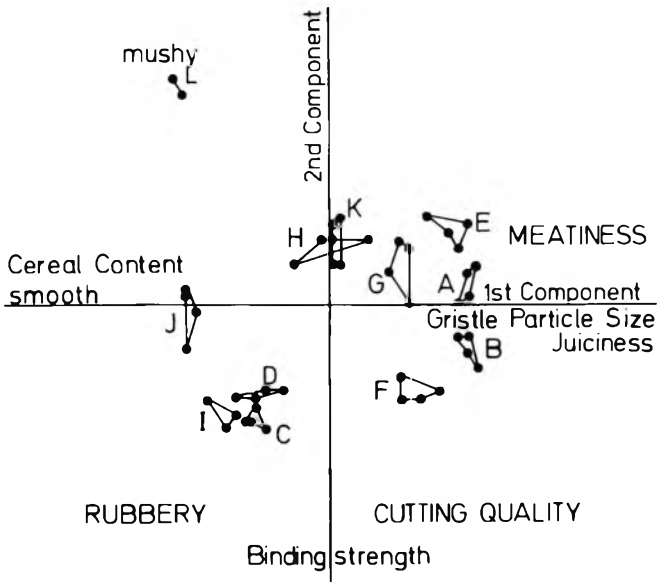

Fig. 1. – Plot of 12 patty types (A-L) relative to the first 2 Principal Components of the consensus configuration obtained by generalized Procrustes analysis of Free-Choice sensory panel data. The 4 replicates of each type are shown joined together. Also plotted are correlations of individual descriptors of texture with the same first 2 Principal Components. (Descriptors with similar meaning appearing together are in upper case, individual descriptors in lower case.)

each patty type used in the FCP panels was tasted under the same conditions (according to design SR22, Bose et al., 1954) 4 times in 8 sessions of 6 samples each using the consensus profile.

The attributes of the consensus profile have been listed in their apparent order of perception (Table 2). The first 2 attributes were determined by pressing with the flat of a fork (rubberiness) and cutting with the side of a fork (adhesion). Once in the mouth the textural attributes listed in Table 2 were perceived/measured at different stages in the mastication process. Rubberiness was defined as an initial perception when first biting into the sample. Crumbliness was regarded as a measure of the ease with which the sample was broken down into particulate material during chewing. Coarseness related to the size and type of particles the sample broke into, while 'chewiness' effectively measured the toughness of particles in the broken-up and partially-masticated sample. During mastication the released moisture contributed to juiciness while greasiness was a measure of the degree of fat or fattiness detected often as a sensation of mouth coating after swallowing. An overall texture acceptability rating was included with the profile.

Consumer study

A sensory testing facility was set up in a local shopping center using portable screens, tables and chairs. Three individual tasting booths were equipped with identical desk lamps to give uniform lighting. When tasting samples, consumers were isolated from each other and from passing traffic, generally in the center. A small screened working area was set up behind the booths to allow allocation and serving of cooked samples. These samples, as described earlier, were cooked at the laboratory and transported to the shopping center.

The 116 consumers were comprised of 57 females and 59 males with 65 aged between 13 and 39 and 51 aged 40 years or more. Outside the sensory testing facility people were approached and, if they used patties, were asked to take part in the survey after filling in a short demographic sheet. All 12 samples were presented to each consumer. Eight different random orders of the 12 samples were cycled for presentation to the total of 116 consumers. Each consumer received 1 sample (appropriately coded) at a time. The consumers registered their opinion of appearance, tenderness, flavor and overall acceptability on 0-100 mm line scales marked very poor (0), poor (25), neither poor nor good (50), good (75) and excellent (100). They were not required to eat all of each sample and were provided paper cups to spit out unwanted sample and water to rinse between samples. Although 12 samples seemed a lot, there were few, if any, complaints and most participants ate the entire sample.

Statistics

Statistical analyses were carried out using a GENSTAT 5 computer package (Payne et al., 1987). For the free-choice profile experiment the data from each of the 12 panelists list of attributes were assembled into matrices of 48 rows (12 patty types assessed at 4 sessions) by 13 columns where the columns represented the maximum number of attributes used by any panelist. Extra columns of zeros were included where necessary to make the number of columns consistent over all assessors. Using these matrices a generalized Procrustes analysis (Gower, 1975) was carried out and a consensus matrix obtained. Principal components analyses were performed on (a) the consensus matrix obtained after generalized Procrustes analysis of the free-choice profile data and (b) on the correlation matrix of the attribute means for the consensus profile. Data from each of the 8 assessors who attended all panel sessions of the consensus profile were also subjected to generalized Procrustes analysis to test for conformity among assessors.

Analysis of variance was used to ascertain treatment effects and, where appropriate, standard errors and hence least significant differences (LSD). Means of consumer data were compared using analysis of variance. In order to analyze individual consumer scores, regression equations were developed for each consumer using the first 2 principal components from the free-choice profile or the consensus profile principal component analysis as independent variables. The coefficients of the 2 variables for the consumers were then plotted to give a PREFMAP plot (MacFie and Thomson, 1983).

RESULTS & DISCUSSION

Sensory measurements

Free choice profile. From the Principal Components Analysis (PCA) on the consensus matrix obtained after generalized Procrustes analysis of the free-choice profile data, the variance accounted for 55.0%, 26.0%, and 7.2% for the first three components, respectively, (total 88.2%). Scores for the first two components for each of the 12 patty types at each of the 4 replicate sessions have been plotted in Fig. 1. There were quite large differences between most of the various patty types and assessors were consistent over the 4 replicates. A simplified correlation plot for the descriptors is also shown in Fig. 1. This plot was constructed using the correlation of an attribute with the first component (x axis) and its correlation with the second component (y axis). Relationships between sensory scores for the 12 patty types and word plot can be found by directly comparing the relative positions of the attributes and samples shown in Fig. 1. Sample L was considered to be mushy while C, D and I were described as rubbery. Samples A, E and G were predominatly beef and were regarded as having more 'meatiness' than the others. Sample J contained the most cereal (Table 1) and was described as having a high cereal content (Fig. 1). The samples were thus distinguished on factors such as meatiness, gristle, juiciness, particle size, smoothness and cereal.

Consensus profile. The principal components analysis plot for the consensus profile sensory data is shown in Fig. 2. As with the FCP results in Fig. 1, there was clearly good spatial separation between the various types except for D, H and K where there was overlap. The principal component coefficients of the various textural attributes (listed in Table 3) showed that the first component, which accounted for 70.2% of the total variance, related to adhesion (apparent), but primarily to mouthfeel attributes such as rubberiness, coarseness and chewiness of particles. The second component (13.3% of total variance) reflected rubberiness (apparent) and adhesion (apparent) contrasting with coarseness, juiciness, greasiness and overall texture. The third component (7.1%) related predominantly to rubberiness (apparent), juiciness and greasiness contrasting with chewiness of particles and coarseness. The position of the attributes relative to the first two principal axes (83.5% of total

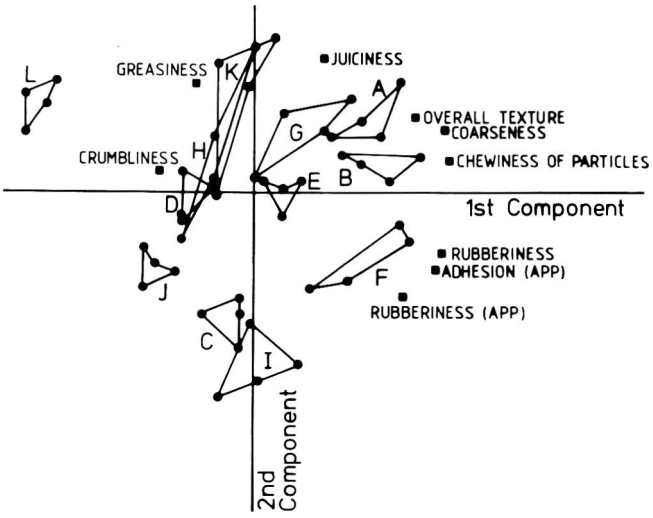

Fig. 2.—Plot of 12 patty types (A-L) relative to the first 2 Principal Components of the consensus profile sensory panel data. The 4 replicates of each type are shown joined together. Loadings of the consensus attributes are also shown for these first 2 axes.

Table 3—Coefficients of components 1, 2 and 3 of the principal components analysis for the consensus profile

Sensory attributes	Principal component coefficients 1	2	3
Rubberiness (Apparent)	0.27	0.41	0.44
Adhesion (Apparent)	0.42	0.40	0.20
Rubberiness	0.37	0.25	0.15
Crumbliness	−0.12	−0.06	0.20
Coarseness	0.48	−0.34	−0.26
Chewiness of particles	0.52	−0.20	−0.32
Juiciness	0.11	−0.48	0.62
Greasiness	−0.08	−0.36	0.38
Overall texture	0.29	−0.31	0.04

variation) are also shown in Fig. 2. The co-ordinates of each attribute were determined by their correlation coefficients relative to the first and second axes.

Generalized Procrustes analyses of the data from each of the 8 assessors who attended all taste panel sessions for the consensus profile showed there was conformity among assessors. Samples had a similar arrangement on the principal axes as for the plot of the samples on the principal components of the consensus profile. The panelists were, therefore, consistent in their use of the consensus profile for assessment of samples.

Flaked (A, B and G) and coarse ground samples were considered to have a coarser structure than fine ground samples (J and L) (Table 1). Samples A, B and F were considered more rubbery and showed greater adhesion than other samples such as K and L. The greasiest sample was L and the least greasy were F, I, and C. F and J did not differ much in juiciness but differed greatly in 'chewiness of particles' and crumbliness. Similarly, I and K did not differ greatly in chewiness of particles and coarseness but differed greatly in juiciness. In terms of acceptability of overall texture, A and B were considered much more acceptable than L or J. The correlations between the various attributes (Table 4) indicated that the attributes assessed with the fork prior to eating were ($P<0.001$) related to rubberiness assessed in the mouth. Rubberiness was ($P<0.001$) related to particle chewiness. The panel, however, obviously had difficulty in differentiating between particle coarseness and particle chewiness ($r = 0.99$, $P<0.001$). Overall texture was best described by coarseness ($r = 0.90$, $P<0.001$) and particle chewiness ($r = 0.84$, $P<0.001$) which highly correlated with each other.

Consumer assessments

The means for all consumer assessments for each of the products have been listed in Table 5. For overall acceptability (which presumably accounted for appearance, tenderness and flavor) best rated samples were K, G and H and the worst rated was E. The K, G and H samples were either flaked or coarse cut, E was a commerical product without spices and flavorings which would normally be included in its formulation. E was considered the worst for flavor and tenderness. In terms of flavor, large and significant differences were found between samples even though similar amounts of flavoring material had been included in formulations.

Each consumer's scores for the 12 patty types on the overall acceptability scale were regressed against the sensory scores of these same 12 products on the first 3 principal components of the consensus matrix of the FCP, as described earlier (statistics section). Similar regressions were also performed for the first 3 principal components of the correlation matrix of the consensus profile. Results have been plotted (PREFMAP)

Table 4—Correlations between the nine textural attributes

Sensory attributes	[a,b]	1	2	3	4	5	6	7	8	9
Rubberiness—App	1	1.00								
Adhesion—App	2	0.89***	1.00							
Rubberiness	3	0.85***	0.97***	1.00						
Crumbliness	4	−0.61*	−0.72**	−0.75**	1.00					
Coarseness	5	0.48	0.69*	0.75**	−0.66*	1.00				
Chewiness of particles	6	0.55	0.78**	0.84***	−0.70*	0.99***	1.00			
Juiciness	7	−0.07	0.01	0.10	−0.51	0.34	0.28	1.00		
Greasiness	8	−0.71**	−0.67*	−0.64*	0.20	−0.37	−0.45	0.46	1.00	
Overall texture	9	0.47	0.55	0.60*	−0.47	0.90***	0.84***	0.42	−0.32	1.00

[a] App = apparent.
[b] Significance of r values; *$P<0.05$, **$P<0.01$, ***$P<0.001$.

Table 5—Consumer results obtained for appearance, tenderness, flavor and overall acceptability of the 12 patty types

Consumer rating[a]	Patty identification[b] A	B	C	D	E	F	G	H	I	J	K	L	LSD[c]
Appearance	43.3	47.7	47.9	51.9	43.6	47.5	56.7	62.5	52.7	64.8	65.6	66.9	5.3
Tenderness	44.4	41.6	62.8	66.8	37.2	42.5	67.1	72.7	40.5	70.7	72.6	76.4	5.2
Flavor	54.6	54.4	49.9	59.3	30.0	49.5	61.4	58.0	39.2	48.7	65.7	53.4	5.9
Overall acceptability	50.0	49.3	50.5	58.0	33.1	46.1	61.8	61.2	39.8	53.6	66.5	57.5	5.5

[a] Consumer rating scored by marking on a 100 mm line with ends labelled very poor (0) and excellent (100) plus ntermediate label of poor (25), neither poor nor good (50) and good (75)
[b] See Table 1 for patty composition and treatment.
[c] Least significant difference at $P<0.05$.

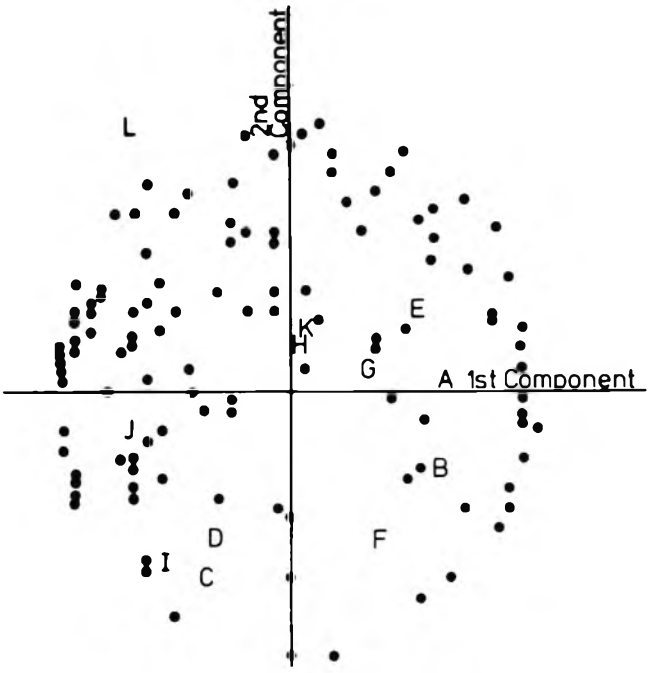

Fig. 3.—Plot of regression coefficients relating overall acceptability data derived form consumers to sensory scores derived from the first 2 principal components of the consensus matrix from the generalized Procrustes analysis. Patty types are labelled A to L as in Table 1.

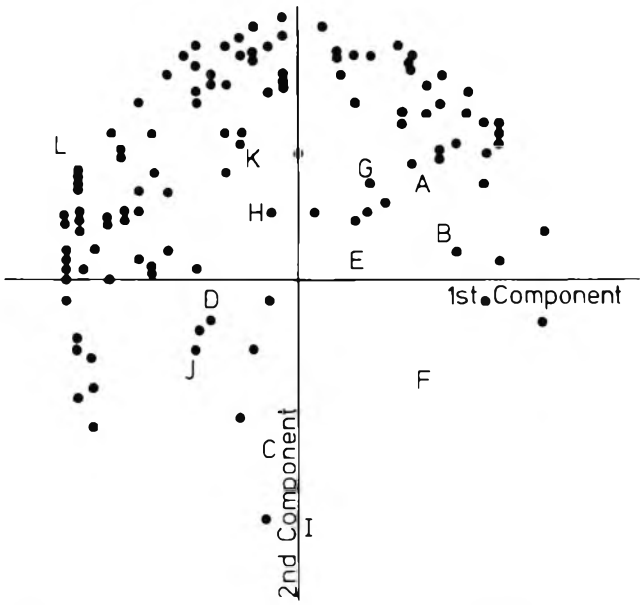

Fig. 4.—Plot of regression coefficients relating overall acceptability data derived from consumers to scores derived from the first 2 principal components of consensus sensory data. Patties are labelled A to L as in Table 1.

in Fig. 3 for the free choice profile and in Fig. 4 for the consensus profile.

The PREFMAP obtained by relating the free choice profile data and consumer data (Fig. 3) showed that the least preferred samples occurred in the lower right quadrant, i.e., samples B and F. These samples would (according to Fig. 1) tend to be high in cutting quality and binding strength. The most preferred samples either had properties akin to "meatiness" or were "mushy." These results were interesting as the consumers clearly liked L which had a texture and consistency

Table 6—Correlations between laboratory sensory attribute data and consumer acceptability ratings

Sensory attribute	Consumer ratings[a]			
	Appearance	Tenderness	Flavor	Overall acceptability
Rubberiness – App.	−0.57	−0.61*	−0.22	−0.40
Adhesion – App.	−0.77**	−0.85***	−0.34	−0.59*
Rubberiness	−0.87***	−0.83***	−0.28	−0.55
Crumbliness	−0.51	0.55	−0.19	0.12
Coarseness	−0.63*	−0.65*	−0.04	−0.27
Chewiness of particles	−0.73**	−0.75**	−0.13	−0.39
Juiciness	0.02	0.16	0.77**	0.58*
Greasiness	0.52	0.52	0.40	0.49
Overall texture	−0.39	−0.38	0.16	0.01

[a] Significance of correlation coefficients at $P<0.05$*, $P<0.01$** and $P<0.001$***.

similar to the low cost patties sold at the local supermarket. In contrast, however, they also liked the high meat content patties such as H, K and G.

The PREFMAP for the consensus profile data (Fig. 4) indicated that the least preferred samples were those in the lower right quadrant, i.e., I and F which were rubbery in texture. The most preferred samples were, (as with the FCP data) L, H, K, G and A. We compared results in Fig. 2, with those in Fig. 4, from the relative positions of the consensus sensory profile attributes. The consumers preferred samples with either coarse, chewy structures which were juicy or which broke up easily over those which were rubbery, i.e., held together or were well bound. There was thus quite good agreement between the two different types of sensory profile (free choice versus consensus) when related to consumer acceptability.

Overall assessment of results

The results obtained using the FCP indicated that the PCA (following the generalized Procrustes analysis) explained 88.2% of the variance using the first three components (55.0, 26.0 and 7.2%, respectively). For the consensus profile about 90.6% of the variance was explained using the first three components (70.2, 13.3 and 7.1%, respectively). There were basically similar patterns of sample configurations relative to the first two components for each of these sensory methods as can be seen in Fig. 1 and 2. Higher discrimination was achieved on the first dimension of the consensus profile relative to the FCP (70.2% vs 55.0%), indicating that there had been some gain in moving from a FCP to a consensus profile. This was to some extent balanced, however, by less variability in the second component of the consensus profile analysis so there was some overlap of samples, i.e. variance was lower at 13.3% than occurred with FCP values of 26.0%.

In essence there was little difference between effectiveness of the two methods. The free-choice profile has the potential to be more useful when looking for differneces in ethnic response to various products since individuals generated their own lists of descriptive words. With a consensus profile the meaning or interpretation of the meaning of each attribute is different languages could be a problem. Overall the consensus profile allowed direct comparisons between different patty types on specific attributes. Such direct comparisons appeared to offer advantage in interpretation when attempting to explain the effects of various treatments on textural properties of manufactured meat products.

The main emphasis of the sensory work in the laboratory was on texture but, although flavoring was added to each patty type to minimize flavor differences, clearly there were important effects of flavor. The consumers were, accordingly, asked to rate samples for flavor as well as for appearance, tenderness and overall acceptability. Relationships between sensory attributes of the consensus profile and consumer ratings (Table 6) indicated that only juiciness related significantly to flavor ($r = 0.77$, $P<0.01$). Flavor and tenderness were, however, both

highly related to overall acceptability (r = 0.94 and 0.86, respectively). Of the individual sensory attributes only adhesion (as determined using the side of a fork) and juiciness related significantly ($P<0.05$) to overall acceptability (Table 6). The consumer acceptability data, as shown in the PREFMAPS (Fig. 3 and 4), demonstrated the directions of increasing consumer acceptance of patties relative to sensory attributes. Demonstrations of such relationships should be useful when attempting to relate sensory attributes to future product consumer acceptance.

Our results have been for a ground meat product, i.e., patties and have shown how sensory techniques such as FCP can be used both alone and as a guide to setting up a consensus profile. The same techniques can be used to generate sensory profiles for other materials such as salami or other, perhaps, more unique types of manufactured meat products. In future work, however, flavor should most likely be included in the profiles since it appeared to be too important to ignore when attempting to predict consumer acceptability.

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Mitochondrial Activity and Beef Muscle Color Stability

M.C. LANARI and R.G. CASSENS

ABSTRACT

Concentration, oxygen consumption rate (OCR), oxygen consumption rate/g meat (OCRM) and metmyoglobin reducing activity (MRA) of muscle mitochondria were determined for beef longissimus dorsi and gluteus medius muscles of Holstein and crossbred steers which differed in color stability. OCR and OCRM decreased but no significant changes were observed in MRA during postmortem storage time. Significant effects of muscle and breed type on OCR, OCRM, and MRA were observed. Muscles and breeds of lower color stability had the highest levels of OCR, OCRM, and MRA. Differences in OCR of muscle mitochondria may be a contributing factor in the effects of muscle and breed on the rate of discoloration.

Key Words: Beef, color, mitochondria, metmyoglobin stability

INTRODUCTION

THE OXIDATION of myoglobin (Mb) to metmyoglobin (Met) has been studied extensively (O'Keefe and Hood, 1982; Hood, 1983; Ledward, 1984; 1985; Renerre and Labas, 1987a). However, the mechanism of Met reduction in meat and the endogenous and exogenous variables that affect it are still not completely understood. Several researchers (O'Keefe and Hood, 1982; Renerre, 1984; Renerre and Labas, 1987a; Faustman and Cassens, 1991) reported that muscle type and breed had a great influence on color shelf life. Renerre and Labas (1987a) and Faustman and Cassens (1991) observed that the gluteus medius (GM) discolored more rapidly than the longissimus dorsi (LD). Faustman and Cassens (1991) indicated that the rate of Met accumulation in Holstein beef was higher than in crossbred meat and suggested that differences in their inherent metabolisms were important factors in meat discoloration.

O'Keefe and Hood (1982) and Renerre and Labas (1987a, b) concluded that the rate of Met accumulation was greatly affected by the tissue oxygen consumption rate. An increase in the oxygen consumption encourages the deoxygenation of oxymyoglobin (Oxy) to myoglobin; Mb is more readily oxidized (Lanari and Zaritzky, 1988) and therefore higher levels of Mb will decrease the shelf life of the product. Ledward (1985) agreed with this while maintaining that differences in the activity of the enzymic reducing system of the tissue were also important in accounting for the variations in color stability among muscles.

Several researchers (Ashmore et al., 1972; Cornforth and Egbert, 1985; Egbert and Cornforth, 1986; Renerre, 1984; Renerre and Labas, 1987b) reported that oxygen consumption by mitochondrial or submitochondrial particles facilitated the maintenance of Mb in the reduced state. Giddings (1974; 1977) proposed that the mitochondria, by reversal of the electron transport chain, may serve as a source of reducing equivalents for extramitochondrial pyridine nucleotide reduction providing the NADH necessary for the functioning of metmyoglobin reductase. Echevarne et al. (1990) reported that the highest Met reductase activity was located in the fraction composed of microsomes and mostly intact mitochondria, but more research was needed to indicate the enzyme systems, which could be soluble or bound to different subcellular fractions. Arihara et al. (1989) reported the presence of a metmyoglobin reductase and identified it as an NADH-cytochrome b_5 reductase. However, in their study the enzyme was purified from beef muscles obtained immediately after slaughter and analysis were not done over an extended post-mortem time course.

The objective of our study was to analyze differences in mitochondrial activity of the color-stable LD and color-labile GM of Holstein and crossbred steers in order to improve understanding of the biochemical factors responsible for variability in color shelf life between muscles and breeds.

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MATERIALS & METHODS

Isolation of mitochondria

Mitochondria from the LD and GM muscles from 4 Holstein and 4 crossbred steers with different post mortem times (3, 48 and 168 hr) at 4°C, were isolated by modifying the method of Ji et al. (1986). Muscles were freed of extracellular fat and connective tissue, sliced and homogenized with a loose fitting Teflon pestle homogenizer. The medium consisted of 250 mM mannitol, 70 mM sucrose, 0.2% free fatty acid albumin and 13 U/mL collagenase (CLS II), pH 7.4. The homogenate was strained twice through one layer of medical gauze to remove remaining fat and connective tissue. After centrifugation at 700 × *g* for 10 min, the supernatant was centrifuged at 12,000 × *g* for 10 min. The final pellets were resuspended in a medium containing 250 mM mannitol, 70 mM sucrose and 1 mM EDTA, pH 7.4. Protein was determined by a biuret method after dissolving the sample in 10% sodium deoxycholate (King, 1967).

Mitochndrial content

The mitochondrial content (mg mitoc prot/g meat) was obtained by measuring the protein content of the muscle, the specific activity of citrate synthase (s.a. cit synt) in isolated mitochondria and in muscle homogenate and then applying the following equation (Idell-Wenger et al., 1978).

$$\frac{\text{mg mitoc prot}}{\text{g meat}} = \frac{\text{mg total prot}}{\text{g meat}} * \frac{\text{s.a. cit synt in muscle homog}}{\text{s.a. cit synt in isolated mitoc}}$$

Citrate synthase was measured after sonication of the Triton X-100 treated samples (0.50 mg Triton/mg protein for mitochondria or 50 mg/g muscle in the homogenate), by the method of Shepherd and Garland (1969). The protein content of the muscle was determined by a biuret method, after digestion of the tissue with 0.05M NaOH (Lilienthal et al., 1950).

Oxygen consumption rate (OCR)

Respiratory properties, expressed as ngr atoms O_2/mg mitoc prot, were followed polarographically with a Clark oxygen electrode at 25°C. The pH of the system was similar to that of the tissue (7.4 for 3 hr postmortem and 5.6 for 48 and 168 hr postmortem). The medium contained 220 mM mannitol, 70 mM sucrose and either 10 mM sodium succinate or 4 mM sodium ascorbate plus 0.4 mM TMPD (tetramethyl-p-phenylene diamine) as substrates; the buffers were 30 mM histidine for pH 5.6 and 15 mM Tris, 5 mM potassium phosphate when the pH was 7.4.

The oxygen consumption rate/g of meat (OCRM) in ngr atoms O_2/min/g of meat was calculated considering OCR values and the amount of mitochondrial proteins/g meat previously obtained.

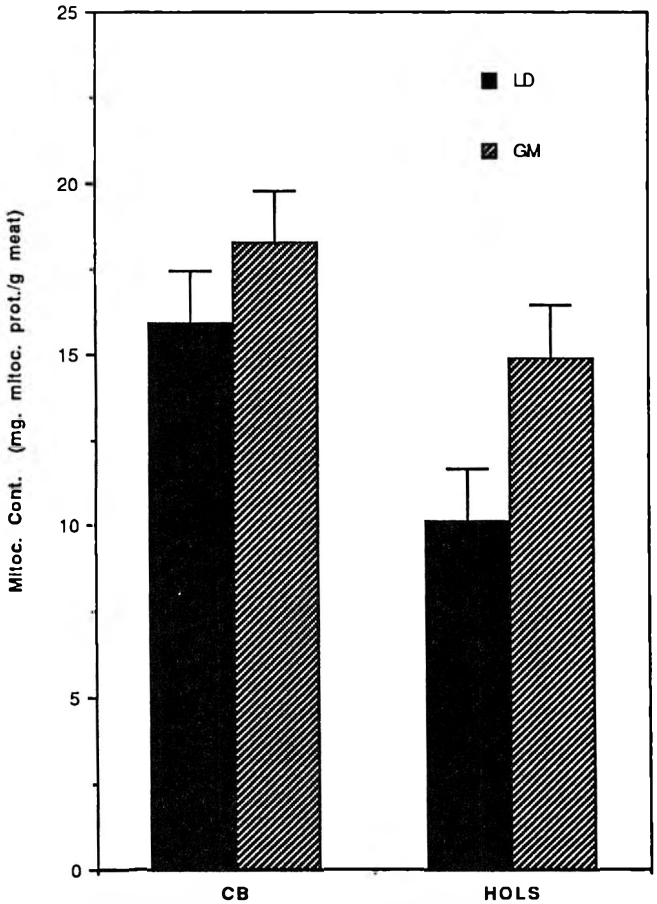

Fig. 1—Mitochondrial content for Longissimus dorsi (LD) and Gluteus medius (GM) of crossbred (CB) and Holstein (HOLS) steers.

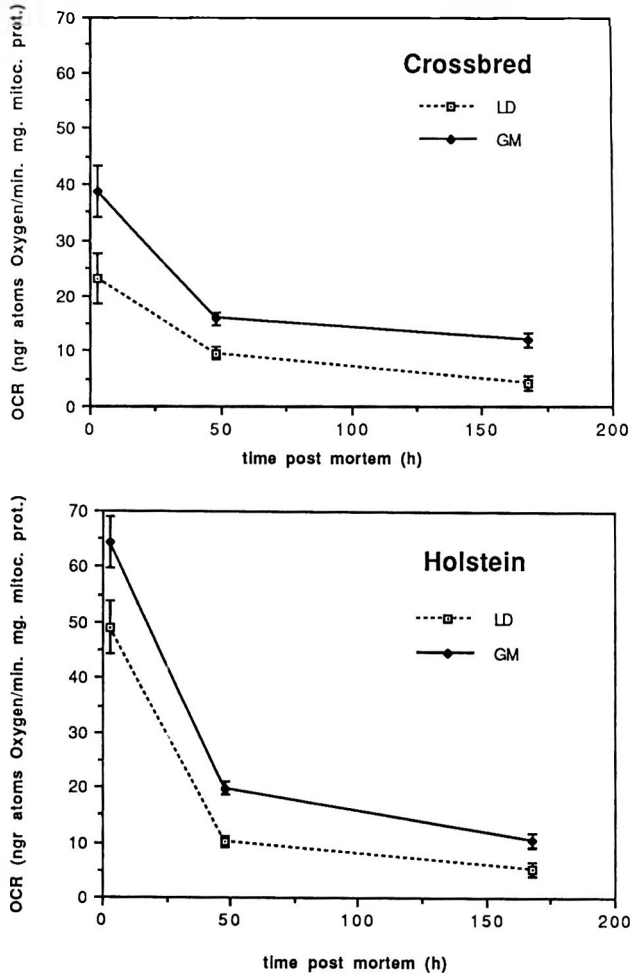

Fig. 2—Time course (hr) of the oxygen consumption rate (OCR) of LD and GM mitochondria from crossbred and Holstein muscle using sodium ascorbate as substrate.

Metmyoglobin reducing activity (MRA)

Metmyoglobin reducing activity was determined at 20°C by a modification of the method described by Renerre and Labas (1987a). The reaction system consisted of: 1.7 mg mitochondrial proteins, 0.065 mmol of horse heart metmyoglobin, 10 μL of 280 μM Methylene blue, 2.65 μmol of NADH and 0.05 μmol rotenone. The buffers were similar to those used in OCR determinations but with no substrate. The reaction was initiated with the addition of NADH and followed at 580 nm (at which the difference between Oxy and Met was maximal). Oxygen uptake by the mitochondria interfered with MRA measurement by favoring the formation of reduced myoglobin. Blocking this reaction required use of an inhibitor (rotenone) of the respiratory chain. Initial rates of reduction (nmol/min/mg mitoc. prot.) were calculated as reported by Faustman et al. (1988).

Statistical analysis

Data were analyzed by the general linear model procedure of SAS (1982) as a split-plot design for the mitochondrial data or a split-split-plot design for OCR or MRA. The animal was designated the main plot, animal × day as the subplot and animal × day × muscle as the sub-sub-plot. Pairwise comparison of means were analyzed by Least Significance Difference tests (SAS, 1982).

RESULTS

Mitochondrial content

Figure 1 shows the mean mitochondrial contents (mg mitoc protein/g meat) for each muscle and breed. Statistical analysis showed that for both breeds, the gluteus medius ($P<0.05$) contained a greater mitochondrial concentration than the longissimus confirming the results of Renerre (1984). When breeds were compared, mitochondrial level in Holstein LD was lower ($P<0.05$) than in crossbred LD and the same was found for the GM.

Several authors (Young, 1982; Renerre, 1984; Renerre and Labas, 1987a) indicated that there was a relationship between meat discoloration and the muscle fiber type composition determined by histochemical and physicochemical measures. Muscles with a high oxidative metabolism have a low color stability. Renerre and Talmant (1981), Renerre (1984) and Renerre and Labas (1987a) reported that the differentiation of muscle metabolic types was more meaningful with mitochondrial content determination than with haeminic iron content measurement or histochemical techniques. Muscles with an elevated mitochondrial content will be highly oxidative and therefore will have a low color stability (Renerre, 1984).

Based on the mitochondrial concentrations previously obtained, metabolism of Holstein LD and GM was less oxidative than the crossbred muscles. In both breeds, the GM was more oxidative than the LD. The muscle comparison confirmed the relationship between color stability and metabolic type. However, our results for breed comparison would not support that hypothesis since Faustman and Cassens (1991) stated that the Holstein muscles were less color stable than the crossbred.

Oxygen consumption rate

Figures 2 and 3 illustrate the effect of postmortem time (TPM) on the respiratory rate of the mitochondria (OCR), using ascorbate plus TMPD (Fig. 2) or succinate (Fig. 3) as substrates. Oxygen uptake was measured using ascorbate and TMPD

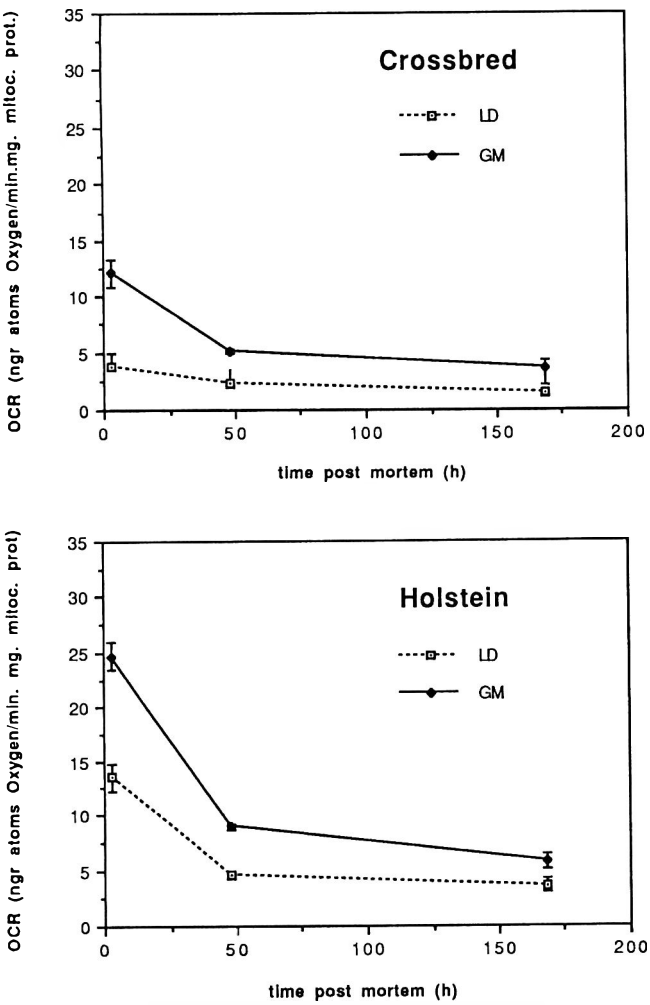

Fig. 3—Time course (hr) of the oxygen consumption rate (OCR) of LD and GM mitochondria from crossbred and Holstein muscle using sodium succinate as substrate.

as substrate because mitochondrial oxidation in its presence is less affected by postmortem time than when succinate is used (Cheah and Cheah, 1974).

Statistical analysis indicated that the postmortem time effect was highly significant ($P<0.0001$) for all times tested (3, 48, 168 hr) using ascorbate as substrate. When succinate was the substrate, the TPM effect was significant ($P<0.0001$) for 3 to 48 hr postmortem, beyond that period further reduction in OCR was not significant ($P>0.05$).

These results were in accordance with those of Ashmore et al. (1972) using mitochondria isolated from triceps brachii, but disagreed with those of Cheah and Cheah (1974). The latter work, with mitochondria from ox neck muscle, found that the decline in OCR with ascorbate after 48 hr storage in situ at 2°C and 10°C was not significant. This disparity could be attributed probably to variations in the rate of decline of tissue pH and to differences in the assay pH. Several workers (Ashmore et al., 1972; Cheah and Cheah, 1974; Giddings, 1974) proposed that a low pH was the main factor in the post-mortem loss of mitochondrial structural integrity and functionality. pH values for LD (Marsh, 1990) and neck muscles (Cheah and Cheah, 1971) at 3 hr postmortem were 6.01 and 6.85 and after 24 hr, 5.6 and 6.2, respectively. The lower initial and final pH and a faster rate of decline in tissue pH of the LD probably produced an inhibitory effect on the respiratory activity of the mitochondria. Cheah and Cheah (1974) determined the OCR using a reaction medium with pH 7.2, for postmortem times of 24 hr or more but this value did not correspond to the actual pH of the muscle. A decrease of the assay pH to values similar to the pH of the tissue (5.6) led to lower respiration rates which better represent postmortem conditions in the muscle.

For all times tested and within each breed, OCR of the GM was higher ($P<0.05$) than that of the LD. When breeds were compared, OCR for LD and GM of Holstein steers were significantly greater ($P<0.05$) than OCR for crossbred muscles confirming the theory that mitochondrial activity depended on metabolic type of muscle. Our results for postmortem changes in OCR disagreed with results of Renerre (1984). However, there were some differences in the protocol used as we measured the OCR of isolated mitochondria at the pH of the muscle (7.2 and 5.6) and he worked with the relationship between the oxygen uptake of muscle homogenate at pH 7.2 and the mitochondrial content.

The OCRM is given in Table 1. For both substrates and during all storage periods (168 hr), the time effect was significant ($P<0.05$). Renerre (1984) measured the OCRM from LD and GM homogenate using pH 7.2, and found that the reduction in oxygen uptake during 192 hr storage at 5°C was very low. This disparity was probably due to the differences in the assay pH.

In spite of having a lower mitochondrial content, Holstein LD and GM had higher OCRM than the crossbred LD and GM respectively (Table 1). These differences were significant ($P<0.05$) for 3 and 48 hr postmortem using ascorbate as substrate. With succinate, the breed effect was significant ($P<0.05$) only in the case of the GM and for 3 hr postmortem. In each breed and for both substrates, OCRM of the GM was greater ($P<0.05$) than for the LD.

Renerre (1984) and Renerre and Labas (1987a) stated that there was an inverse relationship between color stability and oxidative activity indicators such as haeminic iron content, mitochondrial level and OCR. Muscles with the least color stability had the highest oxidative activity indicators. Renerre (1984) reported that the difference in haeminic iron concentration between LD and GM was not significant ($P>0.05$) and therefore their oxygen storage capacity was similar. Results from our study indicated that variability of discoloration between the muscles and breeds could be in part due to changes in OCR since the color labile muscles and breed had the greatest OCRM.

Note that OCR is one of several factors acting together that influence variations in color stability between muscles and breeds. Echevarne et al. (1990) observed that catabolism of NADH was more rapid in unstable muscles such as diaphragma medialis compared with stable muscles such as longissimus dorsi. In their study of differences in biochemical parameters between LD and GM from Holstein and crossbred steers, Faustman and Cassens (1991) reported that the extent of lipid oxidation and the levels of hypoxanthine, glutathione, disulfide and NAD were closely related to discoloration in beef.

Metmyoglobin reducing activity

Results for MRA of the mitochondria from the different muscles and breeds are shown in Table 2. When the reaction was done without methylene blue no reduction was observed. This may indicate that the electron carrier was not present in the mitochondrial fraction and/or that inhibitors of the reaction exist. According to Baseman (1982) low molecular weight substances may act as such inhibitors.

No decrease in reducing activity with time was observed ($P>0.05$). Statistical analysis showed that in each breed, the reducing activity of the GM mitochondria was higher than that of the LD mitochondria ($P<0.05$). Met reductase activity in mitochondria from Holstein LD was greater than in those isolated from crossbred LD ($P<0.05$) and the same was observed for the GM ($P<0.05$). Therefore the color-labile muscle (GM) and breed (Holstein) had the highest reducing activities. These results were consistent with previous reports (O'Keefe and Hood,

Table 1—Muscle and breed effect on the mitochondrial oxygen consumption rate per gram of meat

	OCRMsuc					OCRMasc				
	Holstein		Crossbred			Holstein		Crossbred		
TPM[a]	LD	GM	LD	GM	S.E.	LD	GM	LD	GM	S.E.
R.S.E.										
3	147.45^{ac}	379.86^{b}	53.15^{a}	217.62^{c}	92.36	547.82^{a}	800.81^{b}	326.90^{c}	613.52^{a}	64.74
48	55.95^{ac}	136.28^{b}	29.28^{a}	99.36^{bc}	44.16	339.23^{a}	487.62^{b}	114.32^{c}	259.74^{d}	50.36
168	45.00^{a}	79.09^{a}	25.19^{a}	59.28^{a}	42.99	126.83^{a}	174.62^{a}	81.64^{a}	96.21^{a}	47.24

$^{a-d}$ Values in rows with different superscripts are different ($P<0.05$).

[a] R.S.E. = root mean square error; TPM: time postmortem (hr); OCRMsuc and OCRMasc: oxygen consumption rate using sodium succinate and sodium ascorbate, respectively, ngr atoms O_2/min/g meat.

Table 2—Metmyoglobin reducing activity

	Holstein		Crossbred		
TPM[a]	LD	GM	LD	GM	R.S.E.[a]
3	1.82^{a}	2.94^{b}	1.31^{c}	1.84^{d}	0.18
168	1.50^{a}	2.12^{b}	0.74^{c}	1.46^{d}	0.10

$^{a-d}$ Values in rows with different superscripts are different ($P<0.05$).

[a] R.S.E. = root mean square error; TPM: time postmortem (hr)

1982; Renerre and Labas 1987a; b; Echevarne et al., 1990) but disagreed with the conclusions of Ledward (1985), who (using whole meat) stated that the relative effectiveness of the reducing system governed the rate of formation of Met during storage.

CONCLUSIONS

DIFFERENCES in the OCR of the muscle mitochondria could account in part for the effect of the type of muscles and breed on beef discoloration rate. The effect of mitochondrial MRA in the shelf life of beef is not as important since the least stable muscles and breeds had the highest reducing activities.

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Processing Ingredients Affecting Oxidative and Textural Stability of Restructured Beef Roasts

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ABSTRACT

Restructured beef roasts containing 0.3% sodium tripolyphosphate (STP) and 1% soy protein isolate (SPI); or STP only; or SPI only; or no STP or SPI were cooked at 70 or 100°C to an internal temperature of 65°C, then stored at 4°C for 0 and 3 days. In control roasts, the higher cooking temperature resulted in higher ($p \leq 0.05$) TBA values. STP and SPI inhibited oxidation but the effect was not detected sensorially. Significant interactions ($p \leq 0.05$) indicated that STP was more effective at higher cooking temperatures and SPI at lower temperatures. Total moisture of the cooked product was significantly greater with STP but no differences in juiciness and tenderness were detected sensorially.

INTRODUCTION

A VARIETY of nonmeat protein additives have been used as functional ingredients in restructured meat products. These include various soy proteins used to replace or extend the more costly proteins of meat, poultry and seafood (Endres and Monagle, 1987). Soy protein products have been developed with improved water absorption and binding properties, and could potentially bind red meat during restructuring. Also, they have been demonstrated to have antioxidant activity (Pratt and Birac, 1979) which has been attributed to isoflavones and phenolic acids (Pratt et al., 1981). In a review, Hayes et al., (1977) indicated antioxidant effects of soy proteins carried over into soy concentrates and isolates, but the identity of specific antioxidant compounds was not given.

Phosphates are utilized in restructured meat products to increase water-binding capacity and reduce shrinkage during subsequent processing (Forrest et al., 1975). The addition of phosphates, in the form of pyro-, tripoly-, and hexatema- phosphates (but not orthophosphates), protects cooked meat from oxidative rancidity (Tims and Watts, 1958; Sato and Hegarty, 1971). These phosphates are believed to prevent autoxidation through their ability to sequester heavy metal ions (Tims and Watts, 1958), particularly ferrous iron, which has been shown to be the major prooxidant in meat systems (Love and Pearson, 1974; Igene et al., 1979).

The objectives of our study were to determine the antioxidative effects of both sodium tripolyphosphate and soy protein isolate on restructured beef roasts, evaluate the significance of interactions between these antioxidants and cooking temperature on oxidative stability and determine effects of these ingredients and processing conditions on texture.

MATERIALS & METHODS

Preparation of restructured beef roasts

Beef chucks were obtained through a commercial vendor from carcasses that had not been yield or quality graded. The carcasses were from heifers with Angus-Brahman or Angus-Simmental breeding fed grain for less than 100 days. The lean and fat tissues were separated manually and most connective tissue was removed. Samples were taken from both lean and fat meat blocks for lipid analysis, then all meat was frozen and stored at −18°C until used in roast manufacture. Total frozen storage prior to processing was less than 1 month.

Roasts were manufactured on three separate occasions, representing three replications of the manufacturing process. Required amounts of lean and fat tissue for each replication were thawed at 4°C for 24 hr prior to manufacture. Lean tissues were ground through a 2.54-cm plate and fat tissues through a 0.64-cm plate. Roasts were formulated to contain 10% fat, 10% water, and 0.50% salt. In addition, treatment roasts contained 1% soy protein isolate (SPI, Protein 500E, Ralston Purina, St. Louis, MO); 0.3% sodium tripolyphosphate (STP, Flavorite Laboratories, Inc., Memphis, TN); or a combination of the two. Roasts were prepared by mixing the required amounts of lean tissue, water and salt for 5 min. The fat tissue was added, and mixing was continued for an additional 5 min. SPI was added with the fat, while STP was added along with the salt.

The meat dough was stuffed into a rectangular stainless steel mold (Hoy Equipment Co., Milwaukee, WI), frozen (−18°C) and cooked the following day. Restructured roasts were preweighed and cooked from the frozen-state in a preheated (70 or 100°C) waterbath (Model 1140A-1, Blue M Electrical Company, Blue Island, IL) to internal temperature 65°C. Cook yield was calculated by dividing the weight of the roast after cooking by the weight of the roast prior to cooking, and expressed as percentage of uncooked weight.

The cooked restructured beef roasts were subdivided into 2.54-cm thick slices. Samples from one-half of each roast were immediately vacuum packaged at −1000 millibars (Bizerba Model R2/100, Bizerba Inc., Edison, NJ) in polyethylene (PE) bags and kept frozen (−18°C) until analyzed. Unsealed PE bags with slices from the other half of each roast were stored 3 days at 4°C, vacuum packaged and kept frozen at −18°C until analyzed. Analyses were in a random fashion within analytical procedure and all were completed within 60 days of frozen storage. Sensory and thiobarbituric acid (TBA) procedures were completed within the first week of frozen storage.

Slices used for analyses were thawed overnight in a 4°C cooler. Those for thiobarbituric acid (TBA), total lipid, nonheme and total iron, Instron shear force and sensory analysis were reheated by microwave using 80% of 1450 watts full power (Model CP55, NI Industries, Inc. Los Angeles, CA) to 65°C, as determined by the temperature probe attachment of the microwave. Slices used for expressible moisture and water-binding were not reheated and were analyzed immediately upon completion of thawing.

Moisture analysis

Expressible moisture was determined using the procedure described by Jauregui et al. (1981). Results were reported as weight lost from original sample as percentage of total moisture of each sample. Water-binding was determined by the procedure of Gierhart and Potter (1978). Modification of the procedure involved centrifugation of samples at 800 × *g*. Results were expressed as g water retained/g sample.

Texture measurements

Tenderness of restructured roasts was measured using the Instron Universal Testing Machine (Model 1122) with a Kramer shear attachment. After reheating, slices were cooled at room temperature (23°C) and sliced into three 0.64-cm thick slices from which 2-cm × 2-cm squares were obtained. The machine was set at crosshead and chart speed 50 mm/min with 200 kg full scale load. Results were reported in kg force/g sample.

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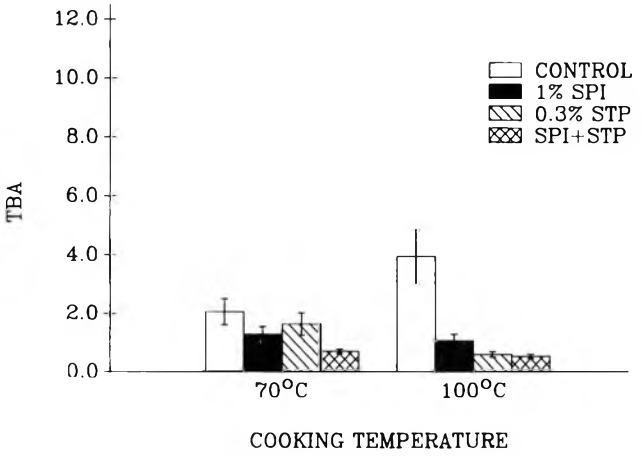

Fig. 1—TBA values of roasts with various levels of antioxidants and two cook temperatures at beginning of refrigerated storage. Soy protein isolate (SPI), sodium tripolyphosphate (STP). TBA in mg thiobarbituric acid reactive substances/kg sample.

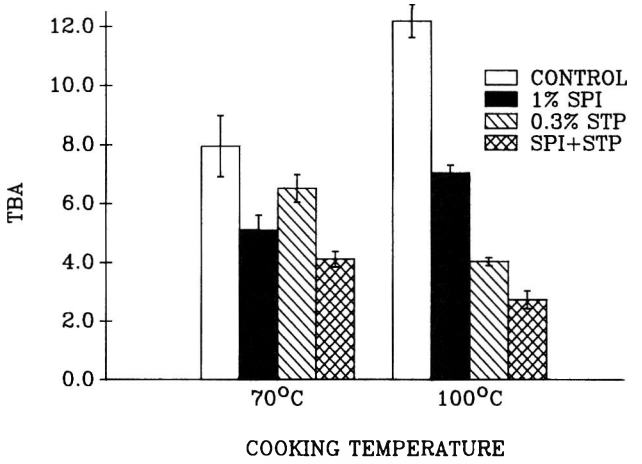

Fig. 2—TBA values of roasts with various levels of antioxidants and two cook temperatures at day 3 refrigerated storage. Soy protein isolate (SPI), sodium tripolyphosphate (STP). TBA in mg thiobarbituric acid reactive substances/kg sample.

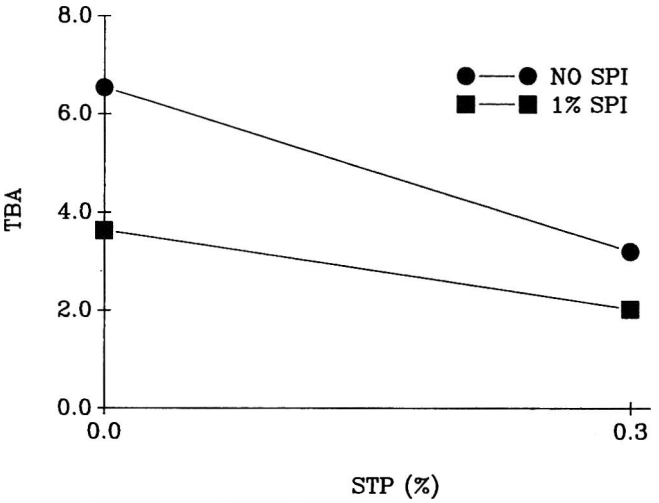

Fig. 3—Interaction between level of sodium tripolyphosphate (STP) and days storage on mean TBA values. Soy protein isolate (SPI), sodium tripolyphosphate (STP). TBA in mg thiobarbituric acid reactive substances/kg sample.

Chemical analysis

The method of Schricker et al. (1982) was used to determine nonheme iron, and total iron was determined by atomic absorption spectrophotometry. Thiobarbituric acid values were determined by the distillation method of Tarladgis et al. (1960) modified by Ockerman (1981). Total lipids were measured by the solvent extraction method described by Kinsella et al. (1977).

Sensory evaluation

Nine graduate students in the Department of Food Science (Louisiana State University, Baton Rouge, LA) who had previously served on sensory evaluation panels of Tanchotikul et al. (1989) and Arganosa et al. (1989) served on this panel. Originally, panelists were trained in five 1-hr sessions. Additional experience was gained by serving on the sensory evaluation panels of previous research projects. Just prior to this study the panelists were refamiliarized with beefy, beany, and warmed-over flavor notes, and tenderness and juiciness in three additional training sessions. Instructions were given to masticate the sample and then expectorate the residue. Tap water was provided for rinsing between samples, as were slices from Red Delicious apples to reduce flavor carryover. In each taste panel session, restructured beef slices were reheated as described previously. The circumferential crust (about 2 mm) was trimmed off before each slice was subdivided into about 1 cm × 1 cm × 2.54 cm rectangular cubes. All cubes were kept warm using a hot sand bath (American Meat Science Association, 1978). Treatments were presented in sets of six with 2 cubes in each souffle serving cup for each treatment over a two-day period for each replication, with a morning and an afternoon session per day. Reheated slices were evaluated using a six-point scale for beefiness (1 = none 6 = strong beefy flavor), warmed-over flavor (1 = strong warmed-over flavor 6 = none), beaniness (1 = strong beany flavor 6 = none), tenderness (1 = very tough 6 = very tender) and juiciness (1 = not juicy 6 = very juicy).

Statistical analyses

The three separate replications of roast manufacture served as blocks in a randomized complete block design with treatments assigned in a split plot arrangement within each block. Cooking temperature, level of STP and level of SPI were main plot treatment factors in a 2 × 2 × 2 factorial arrangement. Storage time was the subplot treatment factor. Data were analyzed using the Statistical Analysis System (SAS, 1985). Analysis of variance was computed by a general linear model procedure. The Duncan Multiple Range Test was used to determine significant differences between means.

RESULTS

Oxidative and flavor aspects

At day 0, control roasts cooked at 100°C had higher ($P \leq 0.05$) TBA numbers than control roasts cooked at 70°C (Fig. 1). Both STP and SPI, as well as the combination of the two, reduced ($P \leq 0.05$) TBA numbers in roasts cooked at 100°C, but only SPI and the combination of the two lowered TBA numbers in roasts cooked at 70°C.

After 3 days refrigerated storage, TBA values increased ($P \leq 0.05$) in all roasts (Fig. 2). The same trends established at day 0 were observed after 3 days storage, with more pronounced differences. Control roasts cooked at 100°C had higher ($P \leq 0.05$) TBA numbers than control roasts cooked at 70°C Also, both additives and the combination lowered TBA numbers in roasts cocked at 100°C. In roasts cooked at 70°C, SPI and the combination reduced ($P \leq 0.05$) TBA numbers. Roasts containing STP had higher ($P \leq 0.05$) TBA numbers when cooked at 70°C, whereas roasts containing SPI had higher ($p \leq 0.05$) TBA numbers when cooked at 100°C. An interaction ($P \leq 0.05$) occurred between SPI and STP (Fig. 3) with regard to TBA number. The combination of STP and SPI produced TBA numbers lower than produced by either additive alone.

A highly significant main effect of storage occurred ($P \leq 0.01$) for both beefiness and warmed-over flavor (WOF), where beefiness declined and WOF increased after 3 days storage. However, panelists distinguished only a few individual differences between treatment-storage combinations (Table 1). No

Table 1—Relation between cook temperature and use of sodium tripolyphosphate (STP) or soy protein isolate (SPi) on sensory and chemical properties of restructured beef roasts stored for 0 or 3 days[a]

Cooking temp = (°C)		0.0% STP				0.3% STP			
		0.0% SPI		1.0% SPI		0.0% SPI		1.0% SPI	
		70	100	70	100	70	100	70	100
	Day								
Sensory Score for beefiness flavor	0	4.54	4.40	4.01	4.51	4.38	4.51	4.20	4.47
	3	3.75	3.36	3.98	3.58	3.90	4.01	3.75	3.9
Sensory score for warmed-over flavor[c]	0	5.67b	5.28ab	5.22ab	5.67b	5.39ab	5.61ab	5.35ab	5.32ab
	3	4.64ab	4.54a	5.02ab	4.64ab	4.65ab	4.94ab	4.81ab	4.40a
Sensory score for beaniness flavor[d]	0	5.40	5.26	4.61	5.19	4.95	5.48	5.12	4.93
	3	5.06	4.97	5.23	4.75	5.16	5.40	5.24	5.38
Total lipid[e]	0	11.73	12.81	11.11	11.86	11.44	10.57	11.45	10.75
Nonheme iron[f]	0	3.55a	6.22c	5.81c	5.85c	7.04c	5.08bc	3.74ab	8.63d
Total iron[g]	0	23.13	25.74	26.33	26.52	25.68	24.39	20.91	24.47

[a] Means within each response parameter with different letters are different (P≤0.05).
[b] 1 = none 6 = strong.
[c] 1 = strong 6 = none.
[d] 1 = strong 6 = strong.
[e] % wet weight.
[f] μg/g sample (wet weight).
[g] μg/g sample (wet weight).

Table 2—Relation between cook temperature and use of sodium tripolyphosphate (STP) or soy protein isolate (SPI) on sensory and physical properties of restructured beef roasts stored for 0 day[a]

Cooking temp = (°C)	0.0% STP				0.3% STP			
	0.0% SPI		1.0% SPI		0.0% SPI		1.0% SPI	
	70	100	70	100	70	100	70	100
Cook yield[b]	71.42b	54.35a	73.67b	57.08a	70.36b	59.33a	73.36b	61.01a
Total moisture[c]	59.13bc	57.43a	58.08b	57.40a	58.87bc	59.20c	60.90a	58.06b
Exp. moisture[d]	55.83c	44.83ab	58.83c	44.00a	57.17c	49.67b	58.17c	45.33ab
Water-binding[e]	0.39ab	0.45b	0.33a	0.42b	0.36ab	0.35a	0.33a	0.43b
Sensory score juiciness[f]	4.13	3.63	4.17	3.48	3.64	4.09	3.49	3.79
Instron shear force value[g]	5.06a	6.08ab	5.59ab	6.38b	5.13a	7.32c	5.22ab	5.68ab
Sensory score for tenderness	4.16	4.21	4.33	3.93	4.15	4.49	4.15	3.96

[a] Means within rows with different letters are different (P≤0.05).
[b] % raw uncooked weight.
[c] % reheated samples.
[d] of total moisture in cooked samples.
[e] g water/g sample.
[f] 1 = not juicy and 6 = very juicy.
[g] kg/g samiple.
[h] 1 = very tough and 6 = very tender.

differences were found for beefiness. For WOF, differences ($P \leq 0.05$) were observed between control roasts cooked at 70°C at 0 day or those with SPI cooked at 100°C at 0 day and control roasts cooked at 100°C at 3 days or those that contained both additives cooked at 100°C at 3 days storage. No differences were observed for beaniness (Table 1).

There were no differences in total lipid or total iron due to treatment (Table 1). A significant three-way interaction occurred for nonheme iron but the only main effect of treatment was for cooking temperature. Higher cooking temperature produced higher nonheme iron values. For control roasts and those that contained both additives, a higher ($P \leq 0.05$) nonheme iron content was found when cooked at 100°C (Table 1). Cooking temperature had no effect in the other two treatment combinations. The highest ($P \leq 0.05$) nonheme iron values were found in roasts that contained both additives cooked at 100°C.

Textural aspects

Cook yield was lower ($P \leq 0.05$) in roasts cooked at 100°C but additives had no effect (Table 2). Total moisture of reheated samples was higher in all roasts cooked at 70°C except for the STP roasts where total moisture was higher in roasts cooked at 100°C. Expressible moisture was also higher in roasts cooked at 70°C. However, roasts that contained STP and were cooked at 100°C had greater ($P \leq 0.05$) expressible moisture than those that contained SPI and were cooked at 100°C. For water binding, differences between roasts cooked at different temperatures were noted ($P \leq 0.05$) only in those that contained SPI or a combination of STP and SPI. For those, water-binding was higher when cooked at 100°C. Sensory panelists, generally, did not detect differences in juiciness (Table 2).

Instron shear force was highest ($P \leq 0.05$) in roasts that contained STP but not SPI cooked at 100°C (Table 2). Roasts with SPI and cooked at 100°C had higher ($P \leq 0.05$) shear force values than control roasts or STP roasts cooked at 70°C. Sensory analyses did not reveal the specific treatment combination differences in tenderness that had been found by Instron analysis.

DISCUSSION

Oxidative aspects

The higher initial TBA values of the roasts without antioxidants cooked at 100°C (Fig. 1) may have been due to increased reaction rate between substrate and catalyst brought about by the higher energy level of the elevated temperature. In addition, higher temperature could result in rupture of cellular membranes, thus exposing more unsaturated components to oxidation (Peng, 1986).

The results at 0 day were contrary to findings of a previous study (Arganosa et al., 1989) where higher temperature re-

sulted in lower initial TBA values. However, Arganosa et al. (1989) found TBA values following storage were greater in roasts that had been cooked at higher temperatures, which we also found (Fig. 2).

The TBA values of all treatments increased after three days refrigerated storage (Fig. 2). A main effect of storage occurred for WOF and beefiness sensory scores but individual differences between storage-treatment combinations were not detected sensorially (Table 1). Previous work (Tanchotikul et al., 1989) also indicated statistically significant differences in oxidation measured by the TBA reaction were not sensorially detectable.

That STP was more effective in reducing TBA numbers at higher cooking temperatures, whereas SPI was more effective at lower temperatures (Fig. 1 and 2) suggested the two antioxidants retarded oxidation by different temperature-dependent mechanisms. STP is believed to act by binding metal ions (primarily iron) and preventing them from acting as oxidative catalysts (Pearson et al., 1977). Antioxidant activity of soy proteins has been attributed to polyphenolic compounds (Pratt and Birac, 1979) that are similar to other compounds, (e.g. tocopherols), that have antioxidant activity as free radical reaction terminators (Labuza, 1971). Hayes et al. (1977) indicated the antioxidant activity of soy-flour was also in soy protein concentrates and isolates. Pratt and Birac (1979) also found considerable antioxidant activity in isolated soy protein, but stated it was due to compounds other than isoflavones. Labuza (1971) pointed out that effectiveness of tocopherols as antioxidants was somewhat temperature dependent, which may also be the case for antioxidant compounds in SPI. One possible explanation for the greater effectiveness of STP at higher temperature could be that catalysts such as iron more readily reacted with STP at higher temperature.

The interaction between STP and SPI (Fig 3) suggested that they have a synergistic effect in reducing TBA during storage. Comparable results have been shown by Tims and Watts (1958), where a combination of ascorbates and phosphates acted synergistically in retarding rancidity. Also, antioxidants were more effective in reducing oxidation during storage in roasts cooked at 100°C than those cooked at 70°C.

Since there were no differences in total lipid and total iron (Table 1), differences in degree of oxidation in various restructured products could not be attributed to those factors. Differences in nonheme iron of the various treatments occurred (Table 1), although interpreting the meaning of those differences is difficult. Previously, we found nonheme iron increased as endpoint internal temperature of restructured roasts increased (Tanchotikul et al., 1989), and there was significant positive correlation between nonheme iron and TBA values. In the present study, higher cooking temperature in control roasts resulted in both higher TBA values and higher nonheme iron. However, in roasts that contained both additives, even though nonheme iron was higher when cooked at the higher temperature, TBA value was lower. Perhaps, an interaction occurred between nonheme iron and the additives that prevent the prooxidative effect.

Previous research (Sato and Hegarty, 1971; Love and Pearson, 1974) has provided evidence that nonheme iron was responsible for rapid oxidation of cooked meat products. Results suggested that the relationship between nonheme iron content and oxidation may be dependent on other nonmeat ingredients. Further research is necessary to clarify the relationships.

Textural aspects

Lower cook yields and correspondingly lower moisture of the cooked samples were obtained at 100°C than at 70°C (Table 2). Myofibrillar proteins denature and coagulate faster at higher cooking temperatures (Laakkonen, 1973). Under these circumstances, maintenance of protein-protein and protein-water interactions is not favored. As a consequence, more water migrates to the surface of the roasts during cooking. Similar results have been found with individual muscles, such as the longissimus dorsi (Taki, 1965) and the semimembranosus (Schock et al., 1970), and with restructured beef roasts (Arganosa et al., 1989). Opposite results were found with sternomandibularis and rectus muscles (Locker and Daines, 1974).

The amount of expressible moisture also was lower in roasts cooked at 100°C, although no differences were found in sensory scores for juiciness or tenderness (Table 2). Coagulation of muscle protein resulted in shrinkage, including thermal shrinkage of collagen fibers in the connective tissue, which subsequently expresses water from the muscle tissue (Schock et al., 1970). Similar results were obtained by Schock et al. (1970) and Arganosa et al., (1989).

The principal effect of treatment on tenderness occurred when a higher Instron shear value was obtained for roasts that contained STP but not SPI cooked at 100°C. That a higher shear value was obtained at higher cooking temperature was not surprising in light of the adverse effect that higher cooking temperature had on cook yield and moisture attributes. The fact that STP treated roasts had highest shear force at 100°C was unexpected because others have found that phosphates reduced shear values in reheated whole-muscle beef roasts (Paterson and Parish, 1988). However, Miller et al. (1986) found phosphate increased shear values in restructured beef steaks, which they attributed to increased binding or cohesiveness. Therefore the phosphate effect on shear value was probably more a function of roast type, i.e. whole muscle versus restructured. Differences in shear value were not detected by our panelists, however. This was also found by Miller et al. (1986) who speculated that because they used a restructured product with similar size reduction and mixing procedures, sensory differences in texture would be minimal. Possibly our panelists were not sensitive enough to detect differences in objective shear values.

This study has demonstrated that oxidation of precooked restructured beef roasts can be reduced by cooking at a lower temperature and with incorporation of antioxidant additives. Significant cooking temperature-antioxidant interactions were observed, which necessitates consideration of cooking temperature used for maximal effectiveness of antioxidant additives.

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Hot-Fat Trimming and Electrical Stimulation Effects on Beef Quality

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ABSTRACT

We evaluated the effects of hot-fat trimming, low-voltage electrical stimulation (ES) and storage time (3, 7 or 14 days) on quality of beef longissimus dorsi, psoas major, biceps femoris and supraspinatus muscles. Prior to chilling, beef carcasses (n = 32) were either subjected to ES or not stimulated before alternating sides were hot-fat trimmed. Temperature decline, pH, sarcomere length and USDA yield and quality grades were obtained on each carcass side. Muscles were removed from each side and evaluated for quality factors related to storage life and sensory analysis. Generally, no adverse effects of hot-fat trimming, with or without ES, or increased storage time, were observed. Results indicate that hot-fat trimming may be practiced at the industry level without affecting overall quality.

Key Words: hot-fat trimming beef, electrical stimulation, sensory evaluation

INTRODUCTION

THE BEEF INDUSTRY has recently found increased demand from consumers for closely trimmed cuts of beef at the retail level (Savell et al., 1986). To meet the demand, many retailers initiated practices of trimming beef cuts to 6.4 mm or less of external fat. Beef packers introduced special lines of boxed beef, designed specifically towards that segment of the market demanding leaner beef.

With this new approach in the way boxed beef is presented, some questions arise concerning maximizing efficiency throughout the slaughter and fabrication process. An alternative to simply trimming more fat from subprimals on fabrication lines is hot-fat trimming, which involves removing subcutaneous fat in excess of 6.4 mm, all the kidney, pelvic and heart fat and all cod or udder fat before chilling. This may increase the efficiency of fat removal, due to the soft and pliable nature of hot fat. Published reports however, suggest that the amount of subcutaneous fat on beef and lamb carcasses plays a role in the subsequent palatability of the meat (Bowling et al., 1977; Smith et al., 1976; Tatum et al., 1982). The insulatory effect of subcutaneous fat has been shown to increase and/or extend postmortem enzyme activity, and reduce or eliminate shortening of myofibrils in beef carcasses, thus increasing palatability of cooked beef (Bowling et al., 1977). Tatum et al. (1982) attempted to determine a reliable method to predict beef palatability using carcass fatness in conjunction with marbling degree. They found that for carcasses with at least "slight" degrees of marbling, 7.62 mm of subcutaneous fat was sufficient to ensure acceptable beef palatability. Hot-fat trimming of carcasses to 6.4 mm of subcutaneous fat could therefore, interfere with subsequent palatability of beef, and thus may be an unsuitable method of producing leaner cuts. Subjecting carcasses to high voltage electrical stimulation may alleviate undersirable effects of hot-fat trimming. Electrical stimulation of beef carcasses has been extensively studied and is a reliable technique for improving beef tenderness (Chrystall and Hagyard, 1976; Smith et al., 1976; Savell et al., 1977; Dutson et al., 1980). Since electrical stimulation is practiced regularly, it is the most likely alternative to problems that may be associated with hot-fat trimming. The objective of our study was to evaluate the effects of hot-fat trimming on beef quality, and to determine if use of electrical stimulation could alleviate possible undersirable effects of hot-fat trimming.

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MATERIALS & METHODS

FIFTY-ONE CATTLE exhibiting an ultrasound fat thickness of 10 mm ($12^{th}-13^{th}$ rib) or more were selected during a slaughter shift at a commercial packing plant. Ultrasound readings were obtained on cattle immediately prior to hide removal using an Aloka model 210 DX linear array ultrasound machine.

Electrical stimulation and hot-fat trimming.

Immediately after slaughter, hide removal and dressing, alternating carcasses were electrically stimulated (68 volts (v), 3 sec on, 3 sec off; 70 v, 2 sec on, 3 sec off; 70 v, 2 sec on, 3 sec off; 70 v, 2 sec on, 3 sec off) prior to being placed into a separate room adjacent to the initial chilling cooler (2–4°C). The stimulator was installed in a plant and used regularly. Alternating sides were then trimmed of subcutaneous fat in excess of 6.4 mm; all kidney, pelvic and heart fat; and all cod or udder fat and fat present in the flank region. Fat was trimmed using Bettcher Whizard knives and hand knives.

Temperature decline

After trimming, both sides were held for 48 h (0°C) in a cooler containing about 1200 other carcasses. A spray chilling system was employed which involved spraying carcasses with a fine mist of water for 30 sec every 5 min. Temperature of the biceps femoris (BF), longissimus dorsi (LD), psoas major (PM) and supraspinatus (SU) muscles was monitored using Koch meat thermometers inserted into the approximate geometric center of each muscle. Temperatures were recorded at about 1, 6, 12 and 24 hr postmortem.

Grading

At 48 hr postmortem, each side was ribbed between the 12^{th} and 13^{th} ribs, and quality and yield grade factors (USDA, 1980) were assigned by a representative of the Standardization Branch of the USDA.

pH and sarcomere length

A 200g sample was removed (48 hr postmortem) from the 12^{th} rib region of the LD of each side, placed in a Whirl-pack bag, packed in dry ice and shipped to the USDA-ARS Agricultural Research Center in Athens, GA. Samples were in transit about 12 hr and upon arrival, were immediately subjected to pH and sarcomere length analysis. The pH was determined by blending 2g raw LD muscle in a test tube containing 25 mL 5 mM iodoacetate and 150 mL KCl. A Brinkman pH meter was used to read pH of the homogenate. Sarcomere lengths were determined according to the method of Cross et al. (1981), modified for use with fresh muscle by blending the sample with 250 mM sucrose (1:8 w/w) to form the crude homogenate. Ten sarcomeres for each of 3 myofibrils (30 total) were measured on each sample.

Fabrication and storage

Thirty-two carcasses were selected from the initial group of 51. Carcasses were selected on the basis of sex and quality grade so that an equal number of steers and heifers, and as close to an equal number as possible of USDA Choice carcasses were stratified within each of the four electrical stimulation/hot-fat trimming treatment combinations. This was done to reduce bias that could have been introduced into a particular treatment group from an unbalanced number of steers or heifers, or from an unbalanced number of USDA Choice carcasses. An attempt was made to use only USDA Choice carcasses; however, 4 of the 16 nonstimulated carcasses and 3 of the 16 stimulated carcasses were USDA Select grade. Beginning at 2 days postmortem and continuing through 5 days postmortem, sides were fabricated into boneless, subprimal cuts, lean trim, fat trim and bone. The same number of sides from each treatment combination were fabricated each day to prevent bias based on dry aging time. The BF, LD, PM and SU muscles were retained from each side and separated into three equal portions by a cut perpendicular to the length of the subprimal. The three portions of each subprimal were individually weighed and vacuum packaged in Cryovac B620 barrier bags (Cryovac Division, W.R. Grace & Co.-Conn., Duncan, SC), heat shrunk, boxed and stored at 2°C. Immediately after fabrication [about 3 days postmortem (PM)] and at 7 and 14 days postmortem, one of the three portions of each muscle was frozen at −30°C. To prevent bias based on location within muscle, equal numbers of the caudal, middle and cranial portions of each muscle were held for each storage interval.

Purge loss and color analysis

Those portions that were held for 7 and 14 days were removed from packaging after the appropriate storage interval, blotted dry and reweighed to determine percentage purge loss. After reweighing, one steak (2.5 cm thick) was removed from each portion, placed on a plastic foam meat tray and overwrapped with polyvinylchloride film. The remaining portion of each muscle was vacuum packaged and heat shrunk exactly as before, boxed and frozen for subsequent sensory and shear force analysis. The overwrapped steak was placed in a single layer in a retail meat case set at 3°C. One thousand lux of incandescent light was left on continuously for 72 h. Each steak was evaluated at 0, 24 and 72 hr by a three-member untrained panel for redness, overall desirability and discoloration. An eight-point rating scale was used to describe redness and overall desirability where 1 = extremely dark brown and undesirable, and 8 = extremely bright cherry-red and desirable. A seven-point scale was used for describing degree of discoloration where 1 = total surface discoloration and 7 = no discoloration. To better reflect changes that occurred in color of steaks, a Minolta 100 CX Chrometer was used to objectively measure meat color using the Hunter color system of L, a and b, where L = lightness, a = redness and b = yellowness.

Sensory and shear force analysis

The remaining portions of each of the four muscles were shipped, frozen, about 2500 km to the Univ. of Georgia Meat Laboratory. Two 2.5 cm thick steaks were obtained from each portion of the LD, PM and SU muscles, while a 5.0 cm thick roast was obtained from each portion of the BF muscle. One steak from the LD, PM and SU muscles was used for sensory analysis and the other for shear force analysis, while both sensory and shear samples were obtained from the single BF roast. Steaks were placed in a 2°C cooler until 3°C was reached. Steaks were then cooked to an internal temperature of 70°C on Farberware Open Hearth Broilers model 450N (Farberware, Kidde Inc., Bronx, NY). Temperature was monitored using a Molytek potentiometer and a copper/iron thermocouple inserted into the geometric center of each steak. The steaks were weighed prior to, and after cooking to determine cooking loss, wrapped in aluminum foil and placed in a 70°C warming oven before being served to panelists. Each panelist was served two 2.5 × 1.3 × 1.3 samples. Roasts from the BF muscle were handled as described above except that an electric convection oven was used instead of broilers. Two 0.65 mm thick slices were removed from the center of each roast, and 1.3 × 0.65 cm samples were cut from each slice to serve to panelists.

An eight-member sensory panel was selected and trained according to procedures outlined by Cross et al. (1978). Since the objective of the study was to determine treatment effects on major muscles of the carcass, not to determine differences between muscles a separate panel was held for each muscle. About six 30-min training sessions were held for each panel during which panelists were served samples of beef steak or roast that varied widely in the parameters to be evaluated. These were: juiciness, first impression tenderness, overall tenderness and flavor intensity using an eight-point scale where 1 = extremely dry, tough, tough and bland; and 8 = extremely juicy, tender, tender and intense. Training was concluded when panelists were in close agreement and all panelists felt comfortable with the scoring system. Panelists sat in booths in an isolated room free from distractions. Green fluorescent lighting was used to eliminate bias based on sample appearance. Each panelist was instructed to eat crackers and drink water between samples to cleanse the palate. Eight samples were served/session with 2 sessions each day.

The steaks from LD, PM and SU muscles to be used for shear force analysis were cooked exactly like those for sensory evaluation and allowed to equilibrate, as was the remaining portion of the BF roast, to room temperature. As many 1.3 × 1.3 cm strips as possible were removed from each steak and roast parallel to muscle fiber orientation. A Warner-Bratzler shearing device was used to shear each strip perpendicular to the muscle fibers. Shear force was reported as kg force required to shear each sample.

Statistical analysis

All data reported reflects the 32 carcasses selected for fabrication. An analysis of variance for split-plot design was used to analyze all dependent variables and their interactions usings the SAS (1985) program. Muscle was not included in the model since it was not the objective of the study to determine differences between muscles, but rather, the effects of HFT, ES and postmortem storage had on muscles in different locations of the carcass. When a main effect or interaction showed a significant F - value, mean separation was accomplished using a pairwise comparison of least-squares means using Fisher's least significant difference test. The predetermined level of significance was $P < 0.05$.

RESULTS & DISCUSSION

THE MEAN VALUES for yield grading factors of the test population were: hot carcass weight, 331.6 kg; actual fat thickness, 17.0 mm; adjusted fat thickness, 17.5 mm; ribeye area, 78.4 cm^2; kidney, pelvic and heart fat, 4.1%; USDA yield grade, 3.9. Thus, carcasses appeared to be of sufficient fatness to elicit a response to hot-fat trimming if in fact, hot-fat trimming would cause a response. The USDA quality grading factors of the test population are presented in Table 1. Note that normally, beef carcasses are presented for grading at or around 24 hr postmortem, while the carcasses we used were not graded until 48 hr postmortem. This may have led to increased marbling scores and possibly, higher USDA quality grades, than would have been found if carcasses had been graded at 24 hr postmortem. Marbling scores and USDA quality grades were not affected ($P < 0.05$) by hot-fat trimming (HFT) or electrical stimulation (ES). Sides that were trimmed exhibited darker, firmer, coarser textured lean and a higher degree of "heat-ring" formation ($P < 0.05$). Stimulation did not significantly affect lean color or firmness, but coarser texture and greater degree of "heat-ring" were evident ($P < 0.05$) on sides which were stimulated. These data conflicted with published findings that ES decreased development of "heat-ring" and improved lean color in beef (Savell et al., 1978; Calkins et al., 1980; McKeith et al., 1980). "Heat-ring" is believed to occur from "ribbing" (separation of the longissimus muscle between the 12^{th} and 13^{th} ribs from grading purposes) carcasses prior to the completion of rigor (Calkins et al., 1980). Electrical stimulation reduces "heat-ring" by accelerating postmortem glycolysis so that rigor is completed before carcasses are ribbed. Most research involving ES has used high voltage (550 v), whereas our study used low voltage (70 v), which may explain why no improvements in lean color were observed. The fact that ES caused greater development of "heat-ring" is not readily explained; however, none of the differences observed for degree of heat-ring, or lean texture were of the magnitude to affect USDA quality grade, and thus may not be of practical importance.

Table 1—Effect of electrical stimulation and hot-fat trimming on USDA quality grading factors[a]

Treatment	Bone maturity	Lean maturity	Overall maturity	Marbling score	Quality grade	Lean color	Lean firmness	Lean texture	Heat ring
No stimulation	166.0[b]	160.0[b]	164.0[b]	261.0[b]	13.2[b]	6.8[b]	7.1[b]	6.6[b]	4.9[b]
Stimulated	161.0[b]	151.0[b]	156.0[b]	242.0[b]	13.0[b]	6.5[b]	6.7[b]	5.6[c]	4.6[c]
SEM[d]	2.6	4.3	2.5	10.6	0.2	0.2	0.2	0.2	0.1
No trimming	–	–	–	250.0[b]	13.1[b]	7.1[b]	6.6[c]	6.6[b]	4.9[b]
Hot-fat trimmed	–	–	–	252.0[b]	13.1[b]	6.2[c]	7.3[b]	5.6[c]	4.6[c]
SEM[d]				10.6	0.2	0.2	0.2	0.2	0.1

[a] Bone, lean and overall maturity: 100 = A^{00}, 150 = A^{50}, 200 = B^{00}, etc.
Marbling score: 100 = $slight^{00}$, 150 = $slight^{50}$, 200 = $small^{00}$, etc.
Quality grade: 10 = Low Select, 13 = Low Choice, 16 = Low Prime.
Lean color: 1 = black, 8 = very bright cherry-red.
Lean firmness: 1 = extremely soft, 8 = extremely firm.
Lean texture: 1 = extremely coarse, 8 = extremely fine.
Heat Ring: 1 = complete heat ring, 5 = no heat ring.
[b,c] Means in the same column within treatment bearing different superscripts are different ($P < 0.05$).
[d] Standard error of the mean.

Table 2—Effect of hot-fat trimming on temperature decline (°C) of longissimus dorsi, psoas major, biceps femoris and supraspinatus muscles of beef carcasses

Treatment	0 hr	6 hr	12 hr	24 hr
Longissimus dorsi				
No trimming	38.2[a]	19.5[a]	12.2[a]	5.5[a]
Hot-fat trimmed	37.7[b]	15.5[b]	6.9[b]	3.4[b]
SEM	0.2	0.2	0.2	0.2
Psoas major				
No trimming	40.2[a]	28.8[a]	20.8[a]	10.8[a]
Hot-fat trimmed	39.3[b]	20.9[b]	11.6[b]	5.0[a]
SEM	0.2	0.2	0.2	0.2
Biceps femoris				
No trimming	39.4[a]	28.3[a]	19.6[a]	10.4[a]
Hot-fat trimmed	39.7[a]	28.3[a]	19.2[a]	9.9[a]
SEM	0.2	0.2	0.2	0.2
Supraspinatus				
No trimming	39.8[a]	24.5[a]	15.8[a]	7.4[a]
Hot-fat trimmed	39.4[a]	22.5[b]	14.4[a]	6.8[a]
SEM	0.2	0.2	0.2	0.2

[a,b] Means in the same column within muscle bearing different superscripts are different ($P < 0.05$).
[c] Standard error of the mean.

Table 3—Effect of electrical stimulation and hot-fat trimming on ultimate pH values and sarcomere length of beef longissimus dorsi muscle

Treatment	pH	Sarcomere length
No stimulation	5.6[a]	1.7[a]
Stimulated	5.6[a]	1.6[b]
SEM[c]	0.01	0.02
No trimming	5.6[a]	1.7[a]
Hot-fat trimmed	5.7[a]	1.6[a]
SEM[c]	0.01	0.02

[a,b] Means in the same column within treatment bearing different superscripts are different ($P < 0.05$).
[c] Standard error of the mean.

The temperature decline data shows HFT caused certain muscles to chill more quickly than the same muscles from untrimmed sides (Table 2). Hot-fat trimming did not affect muscle temperature during the first hour postmortem, except for the PM and LD muscles, which were slightly colder ($P < 0.05$) on trimmed sides. This was, in part, probably due to the fact that the carcasses were in a separate room (2–4°C) rather than the main chilling cooler (0 C) for the first hour following slaughter. This delay may have had slight effects on postmortem changes within muscles, such as accelerated glycolysis and pH decline. However, both trimmed and untrimmed sides were treated in the same manner, thus any effect this delay may have had on the outcome of the experiment was likely insignificant. Hot-fat trimming resulted in lower ($P < 0.05$) temperatures in the LD and PM muscles compared to untrimmed sides during the initial 24 hr chilling period; while the SU chilled faster between 1 and 12 hr, but was not affected ($P < 0.05$) by HFT after a 24 hr chill. The most pronounced effects of HFT on temperature decline was observed in the PM muscle where temperature had declined an additional 9.2°C by 12 hr postmortem. The lack of any significant differences for temperature decline of BF may be due to the relatively small amount of fat that was removed from the exterior of this muscle, and its relatively large mass. Smith et al. (1976) demonstrated that lamb biceps femoris sustained the least response in rate of chilling from the removal of subcutaneous fat, presumably due to its own mass, proximity to other muscles in the hind leg and/or intermuscular fat depostion.

The least-squares means for sarcomere lengths and ultimate pH (48 h) are given in Table 3. Although LD muscle temperature declined more rapidly during the rigor process in trimmed carcasses, no significant differences due to HFT for ultimate sarcomere length of pH were observed. Electrical stimulation caused the ultimate pH of the LD to be higher ($P < 0.05$) than non-stimulated carcasses, which would support the explanation of greater "heat-ring" formation in stimulated sides, although the reason for the higher pH of LD from stimulated sides was not clear. Stimulation also caused sarcomeres to be shorter ($P < 0.05$) than nonstimulated sides.

Percentage weight loss during vacuum storage (purge) for 7 and 14 days, and during cooking are presented in Table 4. No interactions of the main effects of HFT, ES or storage time were apparent, so the data were pooled by main effect for each muscle. Purge loss was not affected ($P < 0.05$) by HFT or ES, but purge loss was increased ($P < 0.05$) as time in storage increased beyond 7 d for each of the four muscles studied. Cooking loss was not affected by HFT, ES or time in storage, with the exception of the BF muscle, which had lower ($P < 0.05$) cooking losses after 7 d storage than after 3 or 14 days storage.

Tables 5 and 6 show the sensory scores, and Table 7 the shear force values, as affected by HFT, ES and time in storage for each of the 4 muscles studied. The only significant interaction of treatments was that of ES and HFT on overall tenderness scores of LD muscle (data not presented in tabular form). This interaction indicated ES had no effect (5.9 vs 5.9) on overall tenderness of untrimmed sides, while ES increased (5.9 vs 6.1) overall tenderness of trimmed sides. The main effect of hot-fat trimming did not affect ($P > 0.05$) juiciness, first impression tenderness, overall tenderness, flavor intensity or shear force values of LD, BF or SU muscles. These data were generally in agreement with those of Johnson et al. (1989), who evaluated the shear force values of several major muscles from beef carcasses that had been either hot-fat trimmed or processed conventionally. Those workers found no effect of hot-fat trimming on shear force values of the adductor, semimembranosus, quadriceps, psoas major, longissimus dorsi, serratus ventralis or triceps brachii muscles. Our study, however, found that the PM from hot-fat trimmed sides had higher shear force values and was rated as being less tender than those from untrimmed sides ($P < 0.05$). Note that both treatments

Table 4—Effect of electrical stimulation, hot-fat trimming and time in storage on purge and cooking loss of beef longissimus dorsi, psoas major, biceps femoris and supraspinatus muscles

Treatment	Purge loss (%)	Cooking loss (%)
Longissimus dorsi		
No stimulation	2.2[a]	17.2[a]
Stimulated	2.6[a]	17.4[a]
SEM[c]	0.2	0.8
No trimming	2.3[a]	17.3[a]
Hot-fat trimmed	2.4[a]	17.3[a]
SEM[c]	0.2	0.8
3 days storage	–	17.8[a]
7 days storage	1.7[b]	17.3[a]
14 days storage	3.0[a]	16.8[a]
SEM[c]	0.2	0.8
Psoas major		
No stimulation	2.2[a]	21.4[a]
Stimulated	2.7[a]	21.4[a]
SEM[c]	0.2	0.8
No trimming	2.3[a]	21.4[a]
Hot-fat trimmed	2.7[a]	21.5[a]
SEM[c]	0.2	0.8
3 days storage	–	21.5[a]
7 days storage	1.8[b]	21.2[a]
14 days storage	3.0[a]	21.6[a]
SEM[c]	0.3	0.9
Biceps femoris		
No stimulation	2.6[a]	17.2[a]
Stimulated	2.7[a]	17.7[a]
SEM[c]	0.2	0.5
No trimming	2.7[a]	17.1[a]
Hot-fat trimmed	2.7[a]	17.8[a]
SEM[c]	0.2	0.5
3 days storage	–	17.9[a]
7 days storage	1.7[b]	16.3[b]
14 days storage	3.7[a]	18.1[a]
SEM[c]	0.2	0.6
Supraspinatus		
No stimulation	3.0[a]	24.6[a]
Stimulated	3.0[a]	24.7[a]
SEM[c]	0.2	0.7
No trimming	3.2[a]	24.3[a]
Hot-fat trimmed	2.8[a]	25.0[a]
SEM[c]	0.2	0.7
3 days storage	–	24.1[a]
7 days storage	2.0[b]	25.3[a]
14 days storage	4.0[a]	24.6[a]
SEM[c]	0.2	0.4

Table 5—Sensory evaluation[a] of beef longissimus dorsi and psoas major muscles as affected by electrical stimulation, hot-fat trimming and storage time

Treatment	Juiciness	First impression tenderness	Overall tenderness	Flavor intensity	
Longissimus dorsi					
No stimulation	5.8[b]	6.3[c]	6.1[c]	5.8[b]	
Stimulated	5.7[b]	6.7[b]	6.6[b]	5.7[b]	
SEM[d]	0.1	0.1	0.1	0.1	
No trimming	5.7[b]	6.4[b]	6.3[b]	5.8[b]	
Hot-fat trimmed	5.8[b]	6.6[b]	6.4[b]	5.7[b]	
SEM[d]	0.1	0.1	0.1	0.1	
3 days storage	5.6[b]	6.0[d]	5.8[d]	5.7[b]	
7 days storage	5.8[b]	6.5[c]	6.4[c]	5.7[b]	
14 days storage	5.8[b]	7.0[b]	6.9[b]	5.8[b]	
SEM[d]	0.1	0.1	0.1	0.1	
Psoas major					
No stimulation	5.8[b]	7.3[b]	7.3[b]	6.2[b]	6.5[b]
Stimulated	5.7[b]	7.4[b]	7.3[b]	6.2[b]	6.5[b]
SEM[d]	0.1	0.1	0.1	0.1	0.1
No trimming	5.7[c]	7.4[b]	7.4[b]	6.2[b]	6.5[b]
Hot-fat trimmed	5.9[b]	7.2[c]	7.2[c]	6.2[b]	6.5[b]
SEM[d]	0.1	0.1	0.1	0.1	0.1
3 days storage	6.0[b]	7.2[c]	7.1[c]	6.3[b]	6.6[b]
7 days storage	5.6[c]	7.3[b]	7.3[bc]	6.2[c]	6.4[c]
14 days storage	5.7[bc]	7.5[b]	7.5[b]	6.1[c]	6.5[bc]
SEM[d]	0.1	0.1	0.1	0.1	0.1

[a] Evaluations expressed on an 8-point scale where 1 = extremely dry, tough, bland and unpalatable; and 8 = extremely juicy, tender, intense and palatable.
[b,c] Means in the same column within muscle and main effect bearing different superscripts are different ($P < 0.05$).
[d] Standard error of the mean.

Table 6—Sensory evaluation[a] of beef biceps femoris and supraspinatus muscles as affected by electrical stimulation, hot-fat trimming and storage time

Treatment	*Juiciness*	*First impression tenderness*	*Overall tenderness*	*Flavor intensity*	
Biceps femoris					
No stimulation	5.5[b]	5.5[b]	5.1[b]	5.2[b]	
Stimulated	5.6[b]	5.7[b]	5.3[b]	5.3[b]	
SEM[d]	0.2	0.1	0.1	0.1	
No trimming	5.5[b]	5.5[b]	5.1[b]	5.3[b]	
Hot-fat trimmed	5.6[b]	5.6[b]	5.2[b]	5.2[b]	
SEM[d]	0.2	0.1	0.1	0.1	
3 days storage	5.5[b]	5.3[c]	4.9[b]	5.1[b]	
7 days storage	5.5[b]	5.7[bc]	5.3[b]	5.2[b]	
14 days storage	5.6[b]	5.9[b]	5.3[b]	5.3[b]	
SEM[d]	0.2	0.2	0.2	0.1	
Supraspinatus					
No stimulation	5.3[b]	5.3[b]	5.0[b]	5.7[b]	5.1[b]
Stimulated	5.4[b]	5.4[b]	5.1[b]	5.7[b]	5.2[b]
SEM[d]	0.1	0.1	0.1	0.1	0.1
No trimming	5.4[b]	5.4[b]	5.1[b]	5.6[b]	5.6[b]
Hot-fat trimmed	5.3[b]	5.3[b]	5.1[b]	5.7[b]	5.7[b]
SEM[d]	0.1	0.1	0.1	0.1	0.1
3 days storage	5.6b	5.1[c]	4.7[c]	5.7[b]	5.0[b]
7 days storage	5.3[bc]	5.4[bc]	5.2[b]	5.7[b]	5.3[b]
14 days storage	5.2[c]	5.5[b]	5.3[b]	5.6[b]	5.1[b]
SEM[d]	0.1	0.1	0.1	0.1	0.1

[a] Evaluations expressed on an eight-point scale where 1 = extremely dry, tough, bland and unpalatable; and 8 = extremely juicy, tender, intense and palatable.
[b,c] Means in the same column within muscle and main effect bearing different superscripts are different ($P < 0.05$).
[d] Standard error of the mean.

received tenderness scores greater than 7.0 (on a 1 to 8 point scale). These data confirmed those of Johnson et al., (1986), who removed kidney and pelvic fat from beef carcasses prior to chilling. They found that while this process caused tenderloin steaks to have higher shear force values, the differences between steaks subjected to fat removal and controls were minor, and probably would not affect overall quality.

Electrical stimulation of carcasses resulted in lower shear force values for LD muscle ($P < 0.05$), but did not affect ($P > 0.05$) the shear force of PM, BF or SU muscles. Sensory evaluation for juiciness, first impression tenderness, overall tenderness or flavor intensity showed that there was no effect ($P > 0.05$) of ES on sensory traits of LD, PM, BF or SU muscles. Savell et al. (1981) suggested that while ES caused increases in tenderness in beef longissimus muscle, this effect may have been negated by postmortem aging of the meat. The largest effect of any treatment on sensory evaluation was time in storage. In general, sensory scores for first impression and overall tenderness for all muscles increased ($P < 0.05$) with increased time in storage. The only effect of time in storage on shear force values occurred in the LD, where a significant decrease in shear force (increased tenderness) was observed with each increase in storage period. The fact that our sensory panel was able to detect differences in tenderness that were not apparent by evaluating Warner-Bratzler shear values, may be due to the type of tenderness measurements made by the sensory panel. Sensory panelists were trained to differentiate between *first impression* (actomyosin effects) and *overall* (background effects) tenderness, while shear values are a measure of *total* (actomyosin and background effects) tenderness.

The visual panel color ratings and Hunter color scores for the LD and PM are presented in Table 8, and for the BF and SU, in Table 9. No significant interactions were detected, thus data reported are pooled by ES, HFT, vacuum storage time (7 vs 14 days) and retail display time (0, 24, and 72 days). Hot-fat trimming improved discoloration scores for the LD and PM, as well as caused the PM to be rated higher for redness and overall desirability ($P < 0.05$). Objective Hunter a values (redness) were not affected by HFT, while LD and PM steaks from

Table 7—Effect of electrical stimulation, hot-fat trimming and storage time on Warner-Braztler shear supraspinatus force values[a] of longissimus dorsi, psoas major, biceps femoris and supraspinatus muscles

Treatment	Longissimus dorsi	Psoas major	Biceps femoris	Supraspinatus
No stimulation	5.6[b]	3.8[b]	6.4[b]	7.1[b]
Stimulated	5.1[c]	3.7[b]	6.6[b]	7.3[b]
SEM[e]	0.1	0.1	0.2	0.3
No trimming	5.4[b]	3.7[b]	6.6[b]	7.3[b]
Hot-fat trimmed	5.3[b]	3.8[b]	6.4[b]	7.0[b]
SEM[e]	0.1	0.1	0.2	0.4
3 days storage	5.8[b]	3.9[b]	6.6[b]	7.7[b]
7 days storage	5.4[c]	3.7[b]	6.4[b]	7.1[b]
14 days storage	4.8[d]	3.6[b]	6.5[b]	6.7[b]
SEM[e]	0.1	0.1	0.1	0.4

[a] Reported as kg force required to shear a 1.3 × 1.3 cm strip.
[b,c,d] Means in the same column within treatment bearing different superscripts are different ($P < 0.05$).
[e] Standard error of the mean.

Table 8—Effect of electrical stimulation, hot-fat trimming, vacuum storage time, and retail display time on visual color scores[d] and Hunter color values[e] of longissimus dorsi and psoas major muscles

Treatment	Redness	Discoloration	Overall desirability	L	a	b
Longissimus dorsi						
No stimulation	7.3[a]	6.7[a]	7.4[a]	45.7[a]	12.1[a]	7.4[a]
Stimulated	6.8[b]	6.3[b]	6.8[b]	46.2[a]	11.2[a]	7.5[b]
SEM	0.1	0.1	0.1	0.2	0.9	0.1
No trimming	6.9[a]	6.3[b]	7.0[a]	46.3[a]	11.9[a]	7.6[a]
Hot-fat trimmed	7.2[a]	6.6[a]	7.2[a]	45.5[b]	12.0[a]	7.3[b]
SEM	0.1	0.1	0.1	0.2	0.2	0.1
Vacuum storage time						
7 days	7.2[a]	6.4[a]	7.2[a]	45.6[a]	13.1[a]	8.7[a]
14 days	6.9[a]	6.5[a]	6.9[a]	46.2[a]	10.8[b]	6.2[b]
SEM	0.1	0.1	0.1	0.2	0.2	0.1
Retail display time						
0 hr	7.6[a]	6.9[a]	7.6[a]	45.3[b]	13.2[a]	7.5[a]
24 hr	7.1[b]	6.5[b]	7.2[b]	45.4[b]	11.5[b]	7.7[a]
72 hr	6.4[c]	6.0[c]	6.4[c]	47.1[a]	11.2[b]	7.1[b]
SEM	0.1	0.1	0.1	0.3	0.2	0.2
Psoas major						
No stimulation	6.3[a]	6.0[a]	6.5[a]	46.0[a]	11.3[a]	7.0[a]
Stimulated	6.2[a]	5.9[a]	6.3[a]	46.0[a]	10.9[a]	6.9[a]
SEM	0.1	0.1	0.1	0.3	0.2	0.1
No trimming	6.0[b]	5.6[b]	6.1[b]	46.8[a]	10.9[a]	7.3[a]
Hot-fat trimmed	6.6[a]	6.3[a]	6.7[a]	45.3[b]	11.4[a]	6.6[b]
SEM	0.1	0.1	0.1	0.3	0.2	0.1
Vacuum storage time						
7 days	6.3[a]	5.9[a]	6.4[a]	46.2[a]	11.9[a]	8.5[a]
14 days	6.2[a]	6.0[a]	6.3[a]	45.9[a]	10.3[b]	5.5[b]
SEM	0.1	0.1	0.1	0.3	0.2	0.1
Retail display time						
0 hr	7.3[a]	6.6[a]	7.2[a]	45.7[a]	12.4[a]	7.2[a]
24 hr	6.0[b]	5.8[b]	6.2[b]	45.8[a]	10.6[b]	7.1[a]
72 hr	5.5[c]	5.6[c]	5.7[c]	46.6[a]	10.3[b]	6.7[b]
SEM	0.1	0.1	0.1	0.4	0.2	0.1

[a,b,c] Means in the same column within treatment bearing different superscripts are different ($P < 0.05$).
[d] Redness and overall desirability scores based on a 1 to 8 point scale where 1 = extremely dark brown or green and undesirable; and 8 = extremely bright red and desirable.
[e] Based on the Hunter color system where L of black = 0, L of white = 100; a of green = −80, a of red = 100; b of blue = −50, b of yellow = 70.
[f] Standard error of the mean.

trimmed sides had lower L and b values, which indicated they were not as brightly colored. Biceps femoris and SU muscles were not affected by HFT, with exception of lower b values for trimmed vs. untrimmed SU. Electrical stimulation did not have an effect on color of PM or SU, however ES resulted in lower scores for redness and overall desirability, and increased discoloration of LD and BF. Hunter a and b values were decreased by ES, which agreed with the scores of the visual panel. Vacuum storage of cuts beyond 7 days prior to retail display resulted in decreasing Hunter a and b values for all 4 muscles studied. All muscles exhibited similar color changes during retail display; becoming less red, more discolored and less desirable. Apparently, HFT did not reduce the amount of time beef retail cuts could be stored in retail display, compared to controls.

Table 9—Effect of electrical stimulation, hot-fat trimming, vacuum storage time, and retail display time on visual color scores[d] and Hunter color values, of biceps femoris and supraspinatus muscles

Treatment	Redness	Discoloration	Overall desirability	L	a	b
Biceps femoris						
No stimulation	6.5[a]	6.0[a]	6.6[a]	47.6[a]	13.1[a]	9.0[a]
Stimulated	6.2[b]	5.6[b]	6.2[b]	47.1[a]	11.9[a]	8.5[b]
SEM	0.1	0.1	0.1	0.2	0.2	0.1
No trimming	6.3[a]	5.8[b]	6.3[a]	47.2[a]	12.4[a]	8.7[a]
Hot-fat trimmed	6.4[a]	5.8[a]	6.4[a]	47.1[b]	11.9[a]	8.8[b]
SEM	0.1	0.1	0.1	0.2	0.2	0.1
Vacuum storage time						
7 days	5.7[a]	5.2[b]	5.7[b]	47.5[a]	12.6[a]	9.7[a]
14 days	7.0[a]	6.4[a]	7.0[a]	47.2[a]	12.4[b]	7.8[b]
SEM	0.1	0.1	0.1	0.2	0.2	0.1
Retail display time						
0 hr	7.8[a]	6.9[a]	7.8[a]	47.7[b]	16.4[a]	10.3[a]
24 hr	6.3[b]	5.8[b]	6.4[b]	46.7[b]	11.6[b]	8.1[a]
72 hr	4.9[c]	4.7[c]	4.9[c]	47.6[a]	9.6[b]	7.8[b]
SEM	0.1	0.1	0.1	0.3	0.2	0.2
Supraspinatus						
No stimulation	7.2[a]	6.7[a]	7.3[a]	48.3[a]	12.1[a]	6.2[a]
Stimulated	6.8[a]	6.3[a]	7.0[a]	44.2[a]	11.9[a]	6.5[a]
SEM	0.1	0.1	0.1	0.3	0.2	0.1
No trimming	7.1[b]	6.6[b]	7.2[b]	48.8[a]	12.2[a]	6.5[a]
Hot-fat trimmed	6.9[a]	6.5[a]	7.0[a]	43.9[b]	11.8[a]	6.2[b]
SEM	0.1	0.1	0.1	0.2	0.2	0.1
Vacuum storage time						
7 days	7.1[a]	6.5[a]	7.2[a]	48.0[a]	12.6[a]	7.5[a]
14 days	7.0[a]	6.6[a]	7.1[a]	44.7[a]	11.3[b]	5.2[b]
SEM	0.1	0.1	0.1	0.3	0.2	0.1
Retail display time						
0 hr	7.7[a]	7.0[a]	7.8[a]	48.8[a]	12.4[a]	6.0[a]
24 hr	7.1[b]	6.4[b]	7.1[b]	48.7[a]	11.1[b]	6.2[a]
72 hr	6.3[c]	6.3[c]	6.4[c]	44.0[a]	12.4[b]	6.8[b]
SEM	0.1	0.1	0.1	0.3	0.2	0.1

[a,b,c] Means in the same column within treatment bearing different superscripts are different ($P < 0.05$).
[d] Redness and overall desirability scores based on a 1 to 8 point scale where 1 = extremely dark brown or green and undesirable; and 8 = extremely bright red and desirable.
[e] Based on the Hunter color system where L of black = 0, L of white = 100; a of green = −80, a of red = 100; b of blue = −50, b of yellow = 70.
[f] Standard error of the mean.

CONCLUSIONS

CARCASSES used experienced a slight delay (1 hr) before entering the initial chilling cooler and were not graded until 48 hr postmortem. This may have affected the outcome, most notably the rate of temperature decline and marbling scores. Note that our results indicate that hot-fat trimming of beef carcasses prior to chilling did not affect the quality, palatability or retail appearance of beef. While hot-fat trimming caused darker, firmer, coarser-textured lean, and a higher degree of heat ring at 48 hr postmortem, USDA quality grades were not altered. Rate of temperature decline was increased by trimming the longissimus, supraspinatus and psoas major muscles, but no appreciable effect of this change on ultimate sarcomere length or pH of longissimus muscle was observed. Neither weight loss during vacuum storage or cooking loss were affected by fat removal. Finally, hot-fat trimming did not alter palatability ratings or shear force values of the 4 muscles studied, except for some minor tenderness decreases of psoas major muscle. In general, electrical stimulation and postmortem aging had no adverse effects on palatability.

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Improvement of Color and Lipid Stability in Beef Longissimus with Dietary Vitamin E and Vitamin C Dip Treatment

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ABSTRACT

The effects of dietary vitamin E supplementation and vitamin C dip treatment on color and lipid stability in longissimus muscle from Holstein and crossbred beef steers were studied during 16 days of display at 4°C. Dietary vitamin E supplementation retarded metmyoglobin formation of the meat and highly suppressed lipid oxidation compared to the control. Holstein longissimus showed higher metmyoglobin formation than crossbred beef longissimus. Dip treatment with a vitamin C solution was effective in maintaining stability of beef color and lipid.

Key Words: beef, color, ascorbic acid, vitamin E, lipid stability, vitamin C

INTRODUCTION

METMYOGLOBIN FORMATION and lipid oxidation of beef cuts are the most important problems in maintaining a stable display of retail meat. The undesirable brown metmyoglobin results from oxidation of the red oxymyoglobin and purple deoxymyoglobin. Various attempts have been made to reduce pigment and lipid oxidation in meats by dietary vitamin E supplementation of poultry (Webb et al., 1972; Marusich et al., 1975; Bartov and Bornstein, 1977; Uebersax et al., 1978; Bartov et al., 1983), pigs (Hvidsten and Astrup, 1963; Tsai et al., 1978; Buckly and Connolly, 1980) and cattle (Faustman et al., 1989a, b). Faustman et al. (1989b) suggested that vitamin E was absorbed by animals and incorporated into cellular membranes where it performs its antioxidant function. However, feeding vitamin E to cattle may be more effective if combined with a water soluble antioxidant, e.g. vitamin C.

Vitamin C, which is a powerful synergist with vitamin E, has been frequently used as an antioxidant in meat processing, but its use in fresh retail meat cuts is not permitted. In only a few reports has color of beef cuts been stabilized by spraying (Costilow et al., 1955), spreading (Mitsumoto et al., 1991) or dipping (Harbers et al., 1981; Okayama et al., 1987) of vitamin C solutions. Harbers et al. (1981) used a 5% vitamin C solution and Okayama et al. (1987) used a 3% solution to dip, but they reported initial discoloration of beef steaks using those concentrations. The 3% and 5% concentrations of vitamin C may have been too high to be effective. Lower concentrations of vitamin C may be more effective for preservation of beef color and lipid following dip treatment.

Holstein meats are about 15% of total beef consumption in the United States (Buege, 1990). Imported beef from unspecified breeds is consumed at 7% (American Meat Institute, 1989), and beef breed meats are the remaining 78% of consumption. Holstein meats are attractive to consumers because they have less intramuscular fat than beef breed meats (Callow, 1962; Zembayashi et al., 1988). However, Holstein beef retail cuts are reported to discolor more rapidly than beef breed meats (Faustman et al., 1989b). This may be due to differences in pigment stability, perhaps due to a breed difference in metmyoglobin reducing ability. A method to prevent metmyoglobin formation of Holstein retail cuts would be very useful for both meat packers and consumers. The objective of our work was to investigate the effects of dietary vitamin E supplementation and vitamin C dip treatment on color and lipid stability in fresh meat cuts from Holstein and beef steers.

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MATERIALS & METHODS

Materials

Longissimus dorsi muscles from eight crossbred beef steers and ten Holstein steers were used in this study. Four beef steers were fed no supplemental vitamin E and the other four beef steers were supplemented with 1200 I.U. vitamin E (α-tocopheryl acetate, Hoffmann-LaRoche, Inc., Nutley, NJ) per animal daily for 67 days. Five Holstein steers were fed no supplemental vitamin E and the other five Holstein steers received supplemental vitamin E for 38 days at a level of 1200 I.U./animal/day. Vitamin E was dispersed in a liquid carrier. Control steers received the same amount of liquid carrier that contained no vitamin E. Cattle were fed a 90% high-moisture corn-10% corn silage diet formulated to contain 0.1 ppm selenium. The ranges of age and live weight at slaughter of crossbred beef steers were 12–13 months of age and 442-529 kg, and those of Holstein steers were 18–24 months of age and 632–680 kg.

The steers were slaughtered at Packerland Pkg. Co., Green Bay, WI, and the left strip loin from each steer was removed at 24 hr postmortem. These sub-primal cuts were then vacuum-packaged and transported to the University of Wisconsin-Madison meat laboratory and stored for an additional 6 days at 4°C.

The vitamin E contents in longissimus dorsi muscles were measured by the method of Cort et al. (1983).

Dip treatment with vitamin C

Longissimus dorsi muscles were sliced into 1 cm thick steaks, and 50 mm diameter pieces were cut from the sliced steaks with a template cutter. Samples were randomly allotted to undipped control or dip treatments with vitamin C solution. Samples were dipped for 20 sec in a solution of 1% L-ascorbic acid (L-ascorbic acid, sodium salt, Sigma Chemical Co., St. Louis, MO) prepared in sterile distilled water, and drained for 10 sec. All samples were individually placed on plastic foam, over-wrapped with PVC film (MW 4, P = 1000-1050 ml / 645 cm^2 / 24 hr at 23°C, Filmco Ind. Inc., Aurora, OH) and continuously displayed under cool white fluorescent lights (2475 lux) at 4°C for 16 days.

Meat color

Surface metmyoglobin and oxymyoglobin percentages were determined on days 1, 4, 7, 10, 13 and 16 by reflectance spectrophotometry (Krzywicki, 1979) using a Shimadzu UV-265 FW spectrophotometer (Shimadzu Scientific Instruments, Inc., Columbia, MD).

Lipid oxidation analysis

TBA (2-thiobarbituric acid) values were measured in samples displayed for 1, 4, 7, 10, 13 and 16 days by the method of Witte et al. (1970). The weight of each sample was measured before blending and

Table 1—Analyses of variance for surface metmyoglobin, oxymyoglobin percentage and TBA value

Source[a]	d.f.	Metmyoglobin MS	Metmyoglobin F-value	Oxymyoglobin MS	Oxymyoglobin F-value	TBA value MS × 10^{-2}	TBA value F-value
VitE	1	4071.4	13.9**	1986.3	10.6**	8008.9	21.3***
Brd	1	2256.1	7.7*	901.3	4.8*	280.0	0.7
Brd × VitE	1	35.6	0.1	0.0	0.0	59.1	0.2
Brd × VitE × Anml	14	292.9	–	186.6	–	376.1	–
VitC	1	921.3	87.1***	684.8	93.0***	401.6	12.8**
VitC × VitE	1	268.8	25.4***	124.7	16.9**	295.5	9.4**
Brd × VitC	1	6.9	0.6	16.8	2.3	0.1	0.0
Brd × VitC × VitE	1	41.9	4.0	9.6	1.3	17.1	0.5
Brd × VitC × VitE × Anml	14	10.6	–	7.4	–	31.4	–
Day	5	2618.4	67.7***	1820.1	64.3***	1278.7	42.4***
VitC × Day	5	141.6	3.7**	125.3	4.4***	37.1	1.2
Brd × Day	5	19.4	0.5	15.1	0.5	30.9	1.0
Day × VitE	5	943.2	24.4***	488.8	17.3***	997.9	33.1***
VitC × Day × VitE	5	65.0	1.7	33.1	1.2	19.1	0.6
Brd × VitC × Day	5	5.9	0.2	5.9	0.2	3.8	0.1
Brd × Day × VitE	5	35.2	0.9	16.6	0.6	9.7	0.3
Brd × VitC × Day × VitE	5	28.1	0.7	15.4	0.5	7.5	0.2
Error	140	38.7	–	28.3	–	30.2	–

[a] VitE: dietary vitamin E supplementation, Brd: breed (and length of the feeding period), VitC: vitamin C dip treatment, Anml: animal. *P<0.05, **P<0.01, ***P<0.001.

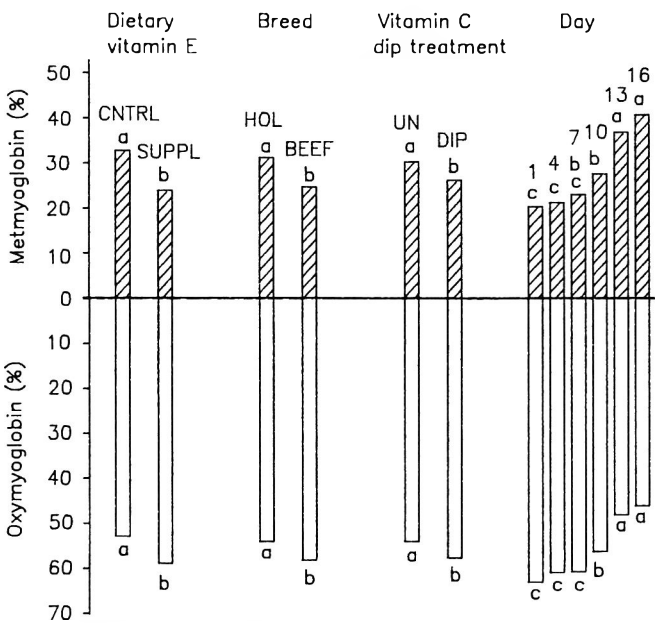

Fig. 1—Least-squares means for surface metmyoglobin and oxymyoglobin percentage. a,b,c: within main effects, means with no common letters differ significantly (P<0.05). CNTRL = no dietary vitamin E supplementation; SUPPL = vitamin E supplementation; HOL = Holstein; BEEF = crossbred beef; UN = undipped control; DIP = dipped in vitamin C solution.

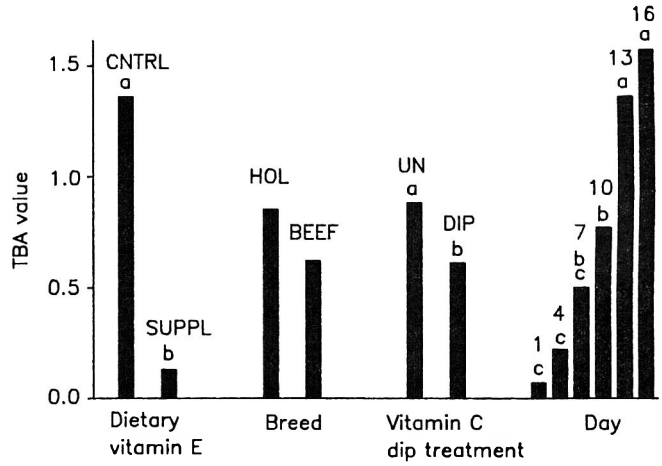

Fig. 2—Least-squares means for TBA value. a,b,c. within main effects, means with no common letters differ significantly (P<0.05). See Fig. 1 legend for treatment codes.

extraction. Trichloroacetic acid solution (20% w/v) was used for extraction. A Beckman DU-65 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA) was used for measuring absorbance at 530 nm. Values of absorbance were multiplied by 5.2 and expressed as mg malonaldehyde equivalent/kg meat.

Statistical analysis

Data were analyzed by the General Linear Models procedure of SAS (1985) as a split-split-plot design to account for the repeated measures aspect. Animal was designated as the main plot, muscle samples within animal as the sub-plot, and muscle samples within animal by day as the sub-sub-plot. Pairwise comparisons of means were analyzed by Scheffe's test (Snedecor and Cochran, 1980). Regression curves of surface metmyoglobin percentages and TBA values versus tissue vitamin E concentrations were computed using statistics library 6 (IBC, 1983).

RESULTS & DISCUSSIONS

THE AVERAGE of α-tocopherol concentration was increased (P<0.01) by vitamin E supplementation in both the crossbred beef steers (control, 2.2; supplemented, 6.0 mg/kg) and Holstein steers (control, 2.2; supplemented, 3.5 mg/kg; SE=0.2). The concentration of α-tocopherol in longissimus of supplemented beef steers was greater (P<0.01) than in longissimus of Holstein steers. Analyses of variance for surface metmyoglobin, oxymyoglobin percentage and TBA value are presented in Table 1, and least-squares means for these measurements are shown in Fig. 1 and 2. Relationships of three-way interactions (vitamin C dip treatment x day x dietary vitamin E) for metmyoglobin and oxymyoglobin percentage and TBA value are presented in Fig. 3-5, because the significant two-way interactions (vitamin C × dietary vitamin E, vitamin C × day, and day × dietary vitamin E) are components of these three-way interactions.

Vitamin E feedings

Dietary vitamin E supplementation to cattle feed greatly improved the stability of meat color and lipid compared to unsupplemented steers. The vitamin E supplemented steers showed lower (P<0.01) metmyoglobin and higher (P<0.01) oxymyoglobin percentages (Fig. 1) and much lower (P<0.001) TBA

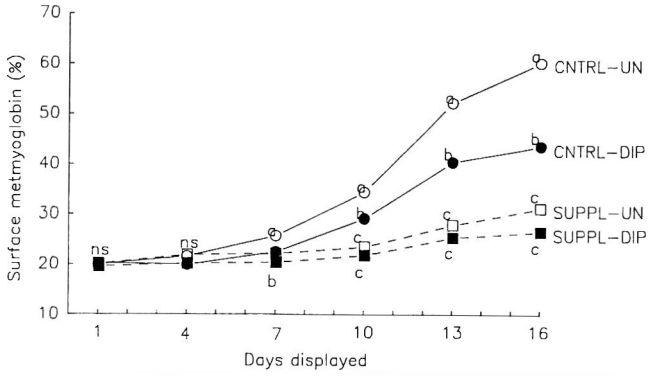

Fig. 3—Relationship of vitamin C dip treatment x days displayed x dietary vitamin E supplementation on surface metmyoglobin percentages. The significance of difference among dietary vitamin E supplementation and vitamin C dip treatment is shown within a day. ns: not significant; a,b,c: means with no common letters differ significantly (P<0.05). CNTRL-UN = undipped control of unsupplemented steers; CNTRL-DIP = vitamin C dip treatment of unsupplemented steers; SUPPL-UN = undipped control of vitamin E supplemented steers; SUPPL-DIP = vitamin C dip treatment of vitamin E supplemented steers.

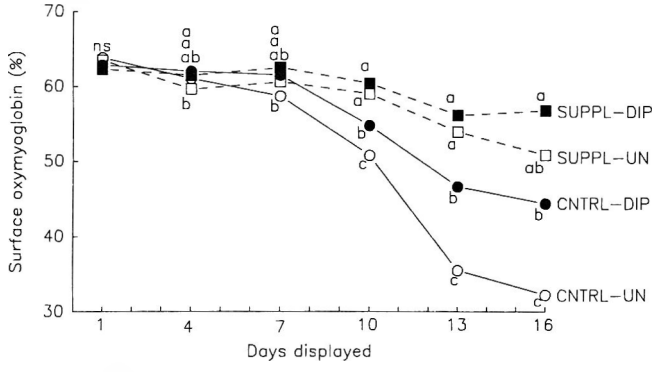

Fig. 4—Relationship of vitamin C dip treatment x days displayed x dietary vitamin E supplementation on surface oxymyoglobin percentages. The significance of difference among dietary vitamin E supplementation and vitamin C dip treatment is shown within a day. ns: not significant; a,b,c: means with no common letters differ significantly (P<0.05). See Fig. 3 legend for treatment codes.

values (Fig. 2) than the unsupplemented steers. Vitamin E supplemented steers had lower metmyoglobin percentages (Fig. 3) from day 7, higher oxymyoglobin percentages (Fig. 4) from day 10, and lower TBA values (Fig. 5) from day 1, than unsupplemented steers. Thus, dietary vitamin E supplementation retarded metmyoglobin formation and greatly suppressed lipid oxidation. Faustman et al. (1989a, b) also reported that vitamin E supplementation (370 I.U./animal/day) of Holstein steers effectively stabilized meat color and lipid of the gluteus medius.

Vitamin E acts as an antioxidant by reacting with free radicals (Tappel, 1962). Green (1969) found that more metmyoglobin reducing activity was retained in ground beef samples stored with an antioxidant. The oxidation in meat is reported to be initiated in the phospholipid-rich membranes (Buckley et al., 1989). Therefore, we suggest that dietary vitamin E was absorbed by steers and incorporated into cellular membranes. In that location vitamin E prevented pigment and lipid oxidation directly by reacting with free radicals and also indirectly maintained metmyoglobin reducing activity. Hence, the stabilities of beef color and lipid were improved.

Relationship between α-tocopherol concentration and, metmyoglobin percentage and TBA value at day 16 is presented in Fig. 6. Supplemented steers (3.2–6.4 mg α-tocopherol/kg)

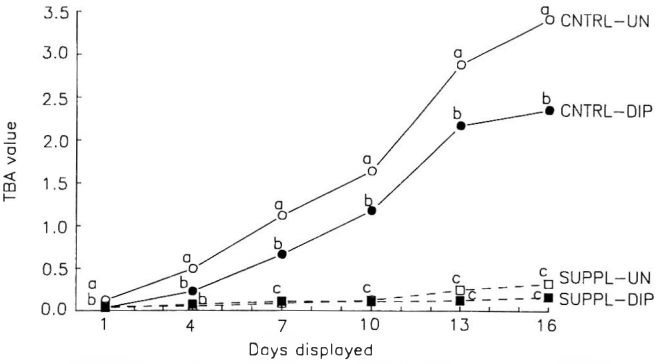

Fig. 5—Relationship of vitamin C dip treatment × days displayed × dietary vitamin E supplementation on TBA values. The significance of difference among dietary vitamin E supplementation and vitamin C dip treatment is shown within a day. ns: not significant; a,b,c: means with no common letters differ significantly (P<0.05). See Fig. 3 legend for treatment codes.

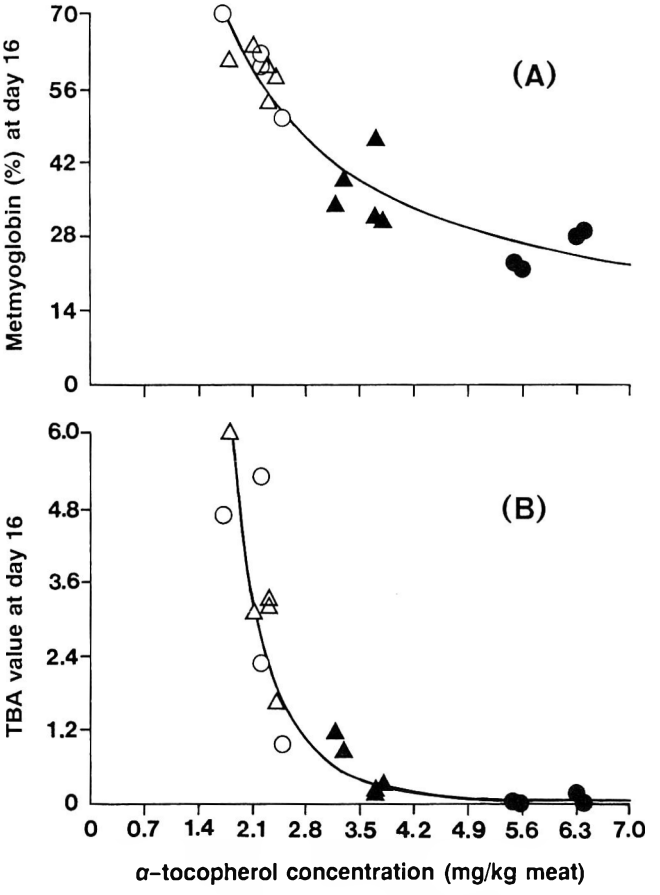

Fig. 6—Relationships between α-tocopherol concentration and, (A) metmyoglobin percentage at day 16 and (B) TBA value at day 16 in undipped control (no vitamin C dip treatment). Regression curves to fit the pooled data (n=18) are indicated. △, control Holstein; ○, control crossbred beef; ▲, vitamin E supplemented Holstein; ●, vitamin E supplemented crossbred beef.

showed lower metmyoglobin percentages and TBA values than control steers (1.7–2.5 mg α-tocopherol/kg), and α-tocopherol concentration over 3.5 mg/kg meat appeared to retard metmyoglobin formation and lipid oxidation. The results indicated that dietary vitamin E supplementation should be maintained at a level to obtain 3.5 mg α-tocopherol per kg meat. Faustman et al. (1989b) also found that Holstein steer beef which con-

tained in excess of ca. 3 mg α-tocopherol/kg tissue diplayed the least oxidation of both pigments and lipids.

Breed effect

Holstein longissimus showed higher ($P<0.05$) metmyoglobin and lower ($P<0.05$) oxymyoglobin percentages than crossbred beef longissimus (Fig. 1), but the TBA values of two breeds did not differ ($P>0.05$; Fig. 2). Faustman and Cassens (1991) reported that Holstein beef had higher values for metmyoglobin percentage, nicotinamide adenine dinucleotide, and TBA, and lower values for glutathione disulfide and hypoxanthine than crossbred beef. In our study, the rate of metmyoglobin formation was higher in longissimus from Holstein than crossbred beef steers. This was consistent with the lower α-tocopherol concentration in longissimus from Holstein than crossbred beef steers. The difference in α-tocopherol concentration was probably due to the shorter period of vitamin E supplementation which the Holstein steers received.

There were no significant ($P>0.05$) interactions between breed and other effects (Table 1). Note that breed effects were potentially confounded by differences in length of the feeding period, age of cattle and weights of the breeds. Holsteins were slaughtered at an older age than crossbreds—a fact which may have influenced myoglobin concentration of muscle.

Vitamin C dip treatment

Dip treatment with vitamin C solution was effective in retarding oxidation of beef color and lipid in comparison with undipped control. Vitamin C dip treatment improved ($P<0.001$) color stability (Fig. 1) and lowered ($P<0.01$) TBA value (Fig. 2) compared to undipped control. Vitamin C dip treatment showed lower metmyoglobin percentages (Fig. 3) from day 7, higher oxymyoglobin percentages (Fig. 4) from day 10, and lower TBA values (Fig. 5) from day 1, than the undipped control.

Tappel et al. (1961) indicated that vitamin C could act synergistically with vitamin E to inhibit lipid oxidation and autoxidation in tissue. The natural vitamin C content of fresh meat is usually considered negligible (0 mg/kg meat; Anderson et al., 1985). On the other hand, the overall mean vitamin E content was 3.4 ± 0.2 mg/kg tissue in the longissimus from the steers in our study. We considered that the vitamin C solution penetrated into the meat to some extent, that it acted as an antioxidant with vitamin E in the meat surface layer and, thus the stability of pigment and lipid was improved.

Harbers et al. (1981) reported that dip treatment of beef psoas major steaks in a 5% vitamin C solution showed an initial discoloration during the first 30 min then slowly increased in brightness in the presence of radiant energy. Okayama et al. (1987) reported that dip treatment of beef short loin steaks in a 3% vitamin C solution showed a higher surface metmyoglobin percentage than controls at day 3 after treatment. In our study, a 1% vitamin C dip treatment showed greater pigment and lipid stability than undipped control during 16 days of display.

Vitamin C dip treatment improved pigment and lipid stability in the steers that were not supplemented with vitamin E but had only a slight effect in vitamin E supplemented steers (Fig. 3–5). Vitamin C penetrated into the meat to some extent after dipping, and acted as an antioxidant with vitamin E in the meat surface layer. However, the overall effectiveness of endogenous vitamin E, especially on lipid oxidative stability, was stronger than that of exogenously added vitamin C (Fig. 2 and 5).

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Near-Infrared Spectroscopy Determination of Physical and Chemical Characteristics in Beef Cuts

MITSURU MITSUMOTO, SATOSHI MAEDA, TADAYOSHI MITSUHASHI, and SHINOBU OZAWA

ABSTRACT

The qualities of beef cuts were compared with near-infrared (NIR) spectroscopy readings using reflectance, transmittance and a fiber optic probe. Multiple linear regression analyses were used to select the optimum wavelengths for estimating beef properties. High multiple correlation coefficients (R) were obtained for Warner-Bratzler shear value (R = 0.798–0.826), protein (R = 0.822–0.904), moisture (R = 0.895–0.941), fat (R = 0.890–0.965) and energy content (R = 0.899–0.961) with each reflectance, transmittance and using the fiber optic probe. Total pigment content also highly correlated with optical densities using transmittance (R = 0.946) and the fiber optic probe (R = 0.893). NIR with a fiber optic probe is a useful tool for determining physical and chemical characteristics of beef.

Key Words: near-infrared spectroscopy, reflectance, fiber-optics, beef quality, optical density

INTRODUCTION

PHYSICAL AND CHEMICAL characteristics of beef including fat content are a matter of concern for both consumers and producers as they influence price as well as nutrient content. Conventional methods of determination are time consuming because samples need to be ground or cooked prior to analysis. Objective measurement of physical and chemical characteristics of beef are therefore not used to assess the quality or value of carcass beef or meat in retail outlets.

Recently, near-infrared (NIR) spectroscopy has been developed as a rapid and accurate technical tool for estimating chemical compositions of foods. There are some reports on meat analysis using NIR spectroscopy. Ben-Gera and Norris (1968) analyzed fat and moisture contents in emulsions of meat products by NIR transmittance. Iwamoto et al. (1981) reported that fat and moisture contents in ground pork correlated highly with NIR reflectance. Kruggel et al. (1981) estimated fat, moisture and protein contents in emulsified beef and ground lamb by NIR reflectance. Lanza (1983) determined moisture, protein, fat and calorie contents in emulsified pork and beef by NIR reflectance and trasmittance. These works were carried out on ground or emulsified meat samples. If the NIR technique can estimate meat characteristics with direct reflectance from raw beef cuts, it would result in considerable time-saving. Swatland (1983) reported that internal reflectance obtained by fiber optics in the 700–1000 nm region of the spectrum correlated with pH value in pork. The analysis of meat pigments in the 400–700 nm region with fiber optics has been reviewed (Swatland, 1989). There are no reports on NIR analysis (680–2500 nm) for physical properties such as cooking loss and shear value or on other chemical characteristics such as total pigment and hydroxyproline content. In addition, the combination of fiber optics with NIR spectroscopy for such analyses should be tested.

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The objective of our work was to evaluate NIR spectroscopy using reflectance, transmittance and fiber optic modes as a means of determining physical and chemical characteristics of beef important to consumers.

MATERIALS & METHODS

Materials

Muscles from eleven Japanese black steers were used in this study. The range of age at slaughter was 22.5–32.4 mo and the range of body weights was 505–681 kg. The following 6 muscles were dissected from the left side of carcasses 48 hr postmortem: semitendinosus, semimembranosus, psoas major, latissimus dorsi, the anterior portion of the longissimus dorsi, supraspinatus. The sample was cut from the center or thickest portion of each muscle and stored for 24 hr at 1°C for subsequent analyses.

Physical and chemical analyses

pH values were determined by directly inserting needle-type glass electrodes into beef cuts. Total pigment contents were determined by the method of Fleming et al. (1960). The total pigment contents were expressed as mg/g wet tissue.

Cooking loss percentages and shear values were measured as follows: steaks (about 2 × 5 × 7 cm) from each sample were placed in a polyethylene bag, and cooked in water at 70°C. Internal temperature of each steak was maintained at 70°C for 30 min. After being cooled to room temperature the sample was weighed and cooking loss determined. The Warner-Bratzler shear values were determined with ten 1.3 cm diameter cores obtained from the above cooked samples. Hydroxyproline levels were measured by the method of Woessner (1961).

Protein contents were estimated from nitrogen content using the Kjeldahl technique and moisture contents were measured by oven drying at 100°C for 16 hours (AOAC, 1984). Fat contents were determined by ether extraction (AOAC, 1984) and energy contents were calculated using energy conversion factors as follows: Energy (Kcal/100g meat) = 4.22 × protein (g/100g meat) + 9.41 × fat (g/100g meat) (Resources Council, Science and Technology Agency, 1982). As the carbohydrate content of beef is very low (0.3–0.7 g/100g meat; Resources Council, Science and Technology Agency, 1982) compared to protein and fat, carbohydrate content was not used in calculation of energy content.

NIR spectroscopy

Reflectance, transmittance and fiber optic spectra measurements were performed by a Neotec Model 6250 Spectrophotometer (NIR-Systems, Inc., Silver Spring, MD). This instrument contains a computer-based system with a single-beam scanning monochromator. The monochromator scans the range 1100–2500 nm in the reflectance mode and the range 680–1235 nm in the transmittance and fiber optic modes. The lead sulfide detector was used for reflectance measurements and the silicon detector was used for transmittance and fiber optic measurements. The fiber optic probe (Fig. 1B) was the surface interactance type (NR-6650VS04X, NIRSystems, Inc., Silver Spring, MD) which had a 20 × 20 mm square sample area consisting of alternating parallel arrays of illuminator and receptor fibers in the ratio of about 3:4. The diameter of a fiber was 0.2 mm, and about 1900 quartz fibers were contained in the total 60 mm^2 illuminator area and 2500 quartz fibers in the total 80 mm^2 receptor area. Data were recorded at 2 nm intervals and 50 scans 25 were averaged for every sample. A ceramic disk was used as reference in each measurement mode. Scanning reference for the fiber optic probe was performed with a

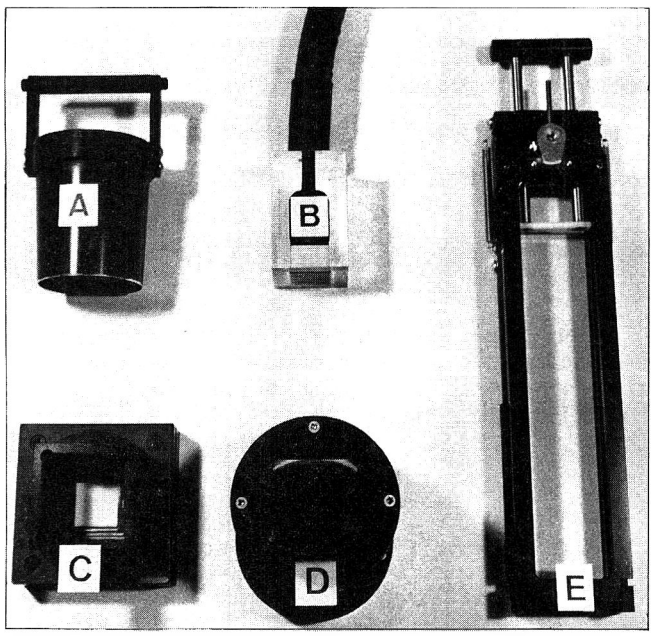

Fig. 1.—Photograph of template cutter (A), fiber optic probe (B), reference cup (C) for fiber optic probe, sample cup (D) for fiber optic probe, quartz sample cell (E) for reflectance and transmittance measurements.

specially designed reference cup (Fig. 1C) which had 2 mm of air distance between the fiber optic probe and ceramic disk.

In reflectance and transmittance modes 1 cm thick steaks were cut perpendicularly to the muscle fiber and placed in polyethylene bags which were then placed in the quartz sample cell (Fig. 1E) and optical densities (O.D.) determined. With the fiber optic probe, a 5.5 cm diam × 6 cm deep sample was cut from the muscle using a template cutter (Fig. 1A) and placed in the specially designed sample cup (Fig. 1D) to prevent interference from outside light. Scannings were performed on both sides of each sample to obtain the average value of individual beef cuts.

Calibration

Data obtained by reflectance and the fiber optic probe were recorded as log $1/R$, where R is the reflectance energy. Absorbance data obtained by transmittance were recorded as log $1/T$, where T is the percent transmittance. Mathematical transformations of the data to second derivatives were computed to reduce effects of differences in particle size and sample composition that can cause baseline offsets.

A multiple linear regression model was used to find the equation which would best fit the data as follows:

$$Y = K_0 + K_1(X_1) + K_2(X_2) + K_3(X_3) + K_4(X_4)$$

where, Y is the constituent percentage or physical value of the characteristics and K_0–K_4 are the constants of the regression equation. X_1–X_4 are the second derivatives of log $1/R$ or log $1/T$ at different wavelengths.

RESULTS & DISCUSSION

MEANS, standard deviations and ranges for physical and chemical characteristics are presented in Table 1. Forty-eight samples were used for calibration with reflectance and 66 samples for calibration with each transmittance and the fiber optic probe. These 66 samples included the 48 samples. Selected wavelengths, correlation coefficients and standard errors of calibration for physical and chemical characteristics by reflectance, transmittance and with the fiber optic probe are presented in Table 2. The second derivative was used as the mathematical transformation in this study. First derivative transformations are recommended for homogeneous products and second derivative transformations are recommended for non-homogeneous products (NIRSystems, 1985). Beef cuts are not homogeneous compared to ground beef. We found that second derivative transformations had slightly better multiple correlation coefficients than first derivative transformations. This observation was in agreement with the report of Lanza (1983).

Table 1—Means, standard deviations (SD) and ranges for physical and chemical characteristics

Item	Mean	SD	Range
Reflectance Mode Set (N = 48)			
pH value	5.57	0.07	5.45–5.76
Total pigment, mg/g	6.01	1.15	3.58–8.20
Cooking loss, %	31.2	4.8	20.4–40.0
Shear value, kg	3.3	1.1	1.7–6.0
Hydroxyproline, mg/g	3.10	1.13	1.10–5.65
Protein, %	18.8	1.3	15.5–20.8
Moisture, %	69.7	4.0	59.6–75.5
Fat, %	8.8	4.9	2.6–22.4
Energy, Kcal/100g	162.6	41.9	103.0–276.2
Transmittance and fiber optic modes set (n=66)			
pH value	5.58	0.08	5.45–5.82
Total pigment, mg/g	5.79	1.27	2.94–8.20
Cooking loss, %	31.0	4.5	20.4–40.0
Shear value, kg	3.5	1.1	1.7–6.0
Hydroxyproline, mg/g	3.11	1.13	0.97–5.65
Protein, %	18.8	1.3	15.0–20.8
Moisture, %	69.7	4.1	58.9–75.6
Fat, %	8.7	5.0	2.6–22.9
Energy, Kcal/100g	161.7	43.1	103.0–278.8

Correlation between O.D. and pH values was highest using reflectance (R = 0.743) compared to transmittance (R = 0.582) and with the fiber optic probe (R = 0.605). Swatland (1983) reported that correlation coefficient for pH value was −0.73 at 700 nm in pork with a fiber optic probe. As every muscle was taken at 48 hr postmortem and kept cool for 24 hr in the same way, the range of pH values (5.45–5.82) of samples was very narrow.

Correlation of O.D. with total pigment content was highest using transmittance (R = 0.946) and the fiber optic probe (R = 0.893), but lower with reflectance (R = 0.639). Total pigment content mainly consists of myoglobin. The vibration of myoglobin should be stronger in the 680–1235 nm region of transmittance and the fiber optic probe than the 1100–2500 nm of reflectance. The first selected 790 nm in the transmittance scans was reported as one of nitrogen-hydrogen overtone bands for protein (Osborne and Fearn, 1986).

Cooking loss correlated highly with O.D. using reflectance (R = 0.771) and transmittance (R = 0.739) compared with fiber optic probe (R = 0.642). Shear value correlated highly with O.D. using transmittance, absorbance and with the fiber optic probe (R = 0.798–0.826) whereas correlations for pH value (R = 0.582–0.743), cooking loss (R = 0.642–0.771) and hydroxyproline content (R = 0.621–0.749) varied considerably between detectors. Shear value had a high negative correlation with taste panel tenderness (Crouse et al., 1978; Culler et al., 1978). These results suggested that NIR technology could predict tenderness of cooked beef from raw fresh beef.

Hydroxyproline content correlated highly with O.D. using reflectance (R = 0.737) and transmittance (R = 0.749) compared with the fiber optic probe (R = 0.621). Hydroxyproline content is used as an index of connective tissue in meat (Lawrie et al., 1964). There were significant relationships between hydroxyproline content and shear value (r = 0.47 and 0.41; P < 0.01, Table 3), between hydroxyproline content and cooking loss (r = 0.65 and 0.69; P < 0.001), as well as between cooking loss and shear value (r = 0.57 and 0.62; P < 0.001). Ozawa (1989) also reported significant relationships between hydroxyproline content and shear value (p < 0.05) and between hydroxyproline content and cooking loss (P < 0.01) in beef. Mitsumoto et al. (1986) reported a significant relationship between cooking loss and shear value in beef (P < 0.05). These observations suggested that similar wavelengths should be selected for cooking loss, shear value and hydroxyproline level.

Table 2—Selected wavelengths and statistical summary for physical and chemical characteristics by the reflectance, transmittance and fiber optic scans

Item	Mode	Wavelength, nm X_1[a]	X_2	X_3	X_4	R[b]	SE[c]
pH value	Reflectance	1188	1508	1332	1964	0.743	0.05
	Transmittance	767	1186	1048	827	0.582	0.07
	Fiber optic	912	763	1117	764	0.605	0.06
Total pigment	Reflectance	2410	2066	1188	1386	0.639	0.93
	Transmittance	790	960	733	1198	0.946	0.42
	Fiber optic	1119	1185	875	955	0.893	0.59
Cooking loss	Reflectance	1332	1958	2422	1562	0.771	3.17
	Transmittance	1200	925	680	958	0.739	3.16
	Fiber optic	1079	955	1082	973	0.642	3.60
Shear value	Reflectance	1414	1740	1884	1830	0.826	0.62
	Transmittance	1177	1081	847	1009	0.798	0.68
	Fiber optic	1084	955	869	761	0.804	0.68
Hydroxyproline	Reflectance	2064	1976	2108	2262	0.737	0.80
	Transmittance	1195	924	1018	1115	0.749	0.77
	Fiber optics	1129	1080	953	1081	0.621	0.91
Protein	Reflectance	1806	2262	2178	2184	0.904	0.58
	Transmittance	941	1014	931	796	0.855	0.71
	Fiber optic	947	1041	992	790	0.822	0.78
Moisture	Reflectance	2322	1760	2156	1388	0.941	1.41
	Transmittance	1184	1057	1139	868	0.940	1.44
	Fiber optic	1058	732	950	1186	0.895	1.89
Fat	Reflectance	2292	1534	1978	1350	0.965	1.34
	Transmittance	890	1056	1141	874	0.965	1.37
	Fiber optic	1000	1057	731	1190	0.890	2.37
Energy	Reflectance	2322	1760	1536	1978	0.955	13.0
	Transmittance	1184	1057	1141	873	0.961	12.3
	Fiber optic	1061	733	950	1122	0.899	19.5

[a] X_1, X_2, X_3, X_4 refer to the selected wavelengths for the linear calibration equation.
[b] Multiple correlation coefficient.
[c] Standard error of the calibration.

Table 3—Correlation coefficients among physical and chemical characteristics[a]

Item	pH value	Total pigment	Cooking loss	Shear value	Hydroxy-proline	Protein	Moisture	Fat	Energy
pH value	—	0.45**	−0.04	−0.26	0.10	−0.53***	−0.32*	0.42**	0.39**
Total pigment	0.26*	—	−0.01	0.03	0.27	−0.28	−0.30*	0.32*	0.31*
Cooking loss	−0.07	0.06	—	0.62***	0.69***	0.14	0.58***	−0.50***	−0.53***
Sheare value	−0.09	0.03	0.57***	—	0.41**	0.39**	0.54***	−0.52***	−0.51***
Hydroxyproline	−0.01	0.22	0.65***	0.47***	—	−0.01	0.34*	−0.26	−0.29*
Protein	−0.36**	−0.27*	0.20	0.40***	0.00	—	0.64***	−0.76***	−0.70***
Moisture	−0.11	−0.15	0.58***	0.56***	0.30*	0.69***	—	−0.98***	−0.99***
Fat	0.20	0.20	−0.50***	−0.55***	−0.23	−0.60***	−0.98***	—	0.996***
Energy	0.17	0.19	−0.53***	−0.55***	−0.25*	−0.75***	−0.99***	0.997***	—

[a] Correlation coefficients in the reflectance mode set (n = 48) are above the diagonal, and those in the transmittance and fiber optic modes set (n = 66) are below the diagonal.
*P < 0.05, **P < 0.01, ***P < 0.001

In this work the two optimal wavelengths using the fiber optic probe were around 1080nm (1079nm, 1084nm and 1080nm, respectively) for cooking loss, shear value and hydroxyproline content, and around 955nm (955nm, 955nm and 953nm).

Protein content correlated highly with O.D. using reflectance (R = 0.904), transmittance (R = 0.855) and with the fiber optic probe (R = 0.822). The selected wavelengths for reflectance (2178 nm and 2184 nm), transmittance (796 nm) and for the fiber optic probe (790 nm) were assigned to nitrogen-hydrogen overtone bands for protein (Osborne and Fearn, 1986). The multiple correlation coefficient for protein in each measurement scan was less than those for moisture and fat. That was due to the variation of protein content in meat being less than variation in moisture and fat contents. The range of protein contents was 5.8% compared with 16.7% for moisture content and 20.3% for fat content. Former workers (Kruggel et al., 1981; Lanza, 1983) also observed that correlation coefficients for protein were not as high as those for moisture and fat in emulsified or ground meat samples. Kruggel et al. (1981) reported multiple correlation coefficients for protein were 0.80 in emulsified beef and 0.67 in ground lamb by NIR reflectance, and Lanza (1983) reported a correlation coefficient of 0.885 for protein in emulsified pork and beef by NIR reflectance. Multiple correlation coefficients for protein reported here for intact muscle tissue were higher or similar to those in ground or emulsified samples reported previously (Kruggel et al., 1981; Lanza, 1983).

Moisture and fat contents correlated highly with O.D. (R = 0.895–0.941 and R = 0.890—0.965, respectively) using transmittance, reflectance and the fibre optic probe. Some selected wavelengths for moisture in the reflectance (1760 nm, 1388 nm), transmittance (1184 nm, 1057 nm) and fiber optic modes (1058 nm, 1186 nm) were similar to identified wavelengths for oil (Williams and Norris, 1987). This suggested that moisture content correlated highly with fat content, and this was confirmed by the high negative r value (−0.98) reported in Table 3. Some specific wavelengths for fat were used in the regression equations for moisture. The selected 1534 nm in reflectance, 874 nm, 890 nm, 1141 nm in transmittance and 1057 nm, 1190 nm with the fiber optic probe for fat were assigned to carbon-hydrogen overtone bands for oil (Osborne and Fearn, 1986). Ben-Gera and Norris (1968) reported correlation coefficients of 0.977 between moisture and ΔO.D. (1800 nm–1725 nm) and 0.974 between fat and ΔO.D. (1725 nm–1650 nm) in 2 mm-thick emulsions of meat products using NIR absorbance. Iwamoto et al. (1981) reported multiple correlation coefficients of 0.972 for moisture and 0.996 for fat in ground pork using NIR reflectance. Kruggel et al. (1981) reported that multiple correlation coefficients were 0.92 for moisture and fat in emulsified beef, 0.70 for moisture and 0.81

for fat, respectively, in ground lamb using NIR reflectance. Lanza (1983) reported that multiple correlation coefficients were 0.987 for moisture and 0.998 for fat in emulsified pork and beef by NIR reflectance. Although our samples were not emulsified or ground, high multiple correlation coefficients for moisture and fat were obtained.

Energy content correlated highly with O.D. with reflectance, transmittance and the fiber optic probe (R = 0.899–0.961). The selected wavelengths for energy were almost the same as those for moisture. This could be expected as fat content more than protein content affects tissue energy content. The high correlation between moisture content and fat content and the similarity between wavelengths selected for moisture and fat support this.

CONCLUSIONS

SHEAR VALUE, protein, moisture, fat and energy contents of beef cuts correlated highly with optical densities from NIR spectroscopy using reflectance and transmittance and with a fiber optic probe. Total pigment content correlated highly with optical densities obtained using transmittance and with the fiber optic probe. NIR spectroscopy could be a useful technique for objectively measuring beef quality and in particular fat, protein and energy contents of beef as well as tenderness during grading and at retail outlets. An NIR spectroscope fitted with a fiber optic probe was almost as efficient as using direct transmittance or reflectance. This flexible fiber optic tool should enable objective measurement of important physical and chemical characteristics of the longissimus dorsi at the site of quartering during beef carcass grading.

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Collagenase Effect on Thermal Denaturation of Intramuscular Collagen

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ABSTRACT

We have investigated the action of a collagenase from *Clostridium histolyticum* on the thermal denaturation parameters (ΔH and initial temperature of denaturation [qt]) of bovine intramuscular collagen. Collagens exhibiting various degrees of reticulation were extracted from calves, steers and cull cows *pectoralis profundis* muscle. Collagenase treatment induced a decrease in the enthalpy of denaturation of calf and steer collagens inversely related to animal age, but no change was observed for cow's collagen. Irrespective of the degree of reticulation, collagen breakdown by the collagenase led to the appearance of another peak of denaturation starting at a lower temperature (55–57°C) than that of intact collagen (62–63°C).

Key Words: collagenase, thermal, denaturation, beef, intramuscular collagen

INTRODUCTION

FOR CONSUMERS, toughness is probably the most critical quality parameter of meat, especially beef. Meat toughness is a complex property depending mainly on the two protein structures which give the muscle its mechanical properties, connective tissue and the myofibrils. Each of the structural components of connective tissue (endomysium, perimysium and epimysium) make a distinct contribution to the overall toughness of meat (Purslow, 1985; Light et al., 1985). The basic collagen structure, known as tropocollagen, consists of three polypeptide chains each twisted in a left handed helix, coiled around each other to form a right handed triple super helix (Aberle and Mills, 1983). As temperature is increased, the collagen's regular structure breaks (denatures) and the chains separate and fold into random structures without any residual native structure.

Collagen is polymerized through the formation of covalent crosslinks. Reducible crosslinks (heat labile aldimine and heat stable keto-imine) are involved in head-to-tail longitudinal crosslinking. This confers considerable tensile strength to the collagen fibers and additional poorly known transverse non-reducible interfibrilar crosslinks which prevent microfibril slippage during mechanical stress (Bailey, 1984).

As assessed through modification of its thermal solubility, changes in the crosslinking state of collagen occur as animals become older, a characteristic markedly affecting meat tenderness (Cross et al., 1972; Reagan et al., 1973; Berry et al., 1974). Heat stability of the crosslinks thus increase with age. This change could be directly characterized through modifications in the energy required for total denaturation (ΔH) and the temperature at which denaturation begins [qt]. These two parameters can be determined from the thermograms of denaturation obtained by differential scanning calorimetry (DSC).

Since the work of Heyns and Legler (1960), the degradation of collagen by a collagenase from *Clostridium histolyticum* is well known. This proteinase degrades the collagen molecule in the helical region, predominantly at the level of the bond Y-Gly in sequences of the type -Pro-Y-Gly-Pro-, where Y is most frequently a neutral amino acid (Nagai, 1961). We investigated the effects of a commercial collagenase from *Clostridium histolyticum* on the denaturation behavior of collagens exhibiting a different degree of reticulation. Our objective was to determine the denaturation parameters, total enthalpy of denaturation (ΔH) and starting temperature of denaturation [qt], from the thermograms obtained by DSC.

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MATERIALS & METHODS

WE USED intramuscular connective tissues extracted from pectoralis profundis from calves, steers and cull cows, animal ages 3 mo to 8 yr. *Clostridium hustolyticum* collagenase (type 1A, EC 3.4.24.3) was obtained from Sigma Chemical Co. (St. Louis, MO).

Intramuscular collagen preparation

Extraction of collagen was according to the method of Kopp et al. (1989). Briefly, muscles trimmed of external lipids and epimysium were ground with a slide grinder, suspended in cold tap water (100 g/L) and homogenized for 30 sec at medium speed in a Waring Blendor. The homogenate was filtered through a graded grid with 1 mm square holes and rinsed, the retained material was rehomogenized and filtered again. This process was repeated two more times, the debris was recovered, defatted with acetone and air-dried. The product comprised endomysial and perimysial collagen with various levels of glycoproteins and elastine. Collagen content was measured by hydroxyproline determination according to the method of Bergman and Loxley (1970) adapted to an auto-analyzer (Bonnet and Kopp, 1984). (Collagen content calculated by using a correcting factor of 7.5 was about 50% of dry matter).

Thermal solubility of collagen

According to Kopp and Bonnet (1982), collagen samples (50 mg) were heated for 6 hr at 90°C in 0.02M Tris-HCl buffer pH 7.5 containing 0.23M NaCl. After filtration, hydroxyproline content of the insoluble fraction was determined as described above for total collagen. Thermal solubility was defined as the difference between total and insoluble collagen concentrations and expressed as a percentage of total collagen.

Differential scanning calorimetry (DSC)

Calorimetry experiments were performed according to the method of Kopp et al. (1989) with a DSC 111 SETARAM (Lyon, France)

Table 1—Denaturation characteristics and thermal solubility of native intramuscular collagen from pectoralis profundis *obtained from cattle of different ages*

Animal type	Age	Collagen (%)	Solubility (% total collagen)	(ΔH) J/g	[qt] °C
Calves	3 mo	42.6±0.6	30.3	58.9±2.8	61.9±0.2
	3 mo	46±1.1	28.2	54.5±2.9	62.7±0.4
Steers	20 mo	52.9±4.8	17.2	42.6±2.8	64.3±0.3
	30 mo	48.4±1.1	12.6	46.5±0.7	64.7±0.6
Cull	5 yr	52.6±0.6	10.6	37.6±4.7	64.8±0.3
Cows	8 yr	63.6±5.3	7.8	41±2.8	65.5±0.3

Table 2—Temperature and denaturation enthalpy change of control and collagenase treated intramuscular collagen from calves, steers and cull cows pectoralis profundis *determined by differential scanning calorimetry*[a]

Animal type	Sample	ΔH (J/g MS)			[qt] [b](°C)	
		Peak 1	Peak 2	Σ	Peak 1	Peak 2
Calves	control	–	67.1 ± 6.1	67.1 ± 6.1[d]	–	57.1 ± 0.6
	treated	10.8 ± 1.9	4.9 ± 0.7	15.7 ± 2.6[h]	53.9 ± 0.4	
Steers	control	–	42.4 ± 1.9	42.4 ± 1.9[e]	–	59.8 ± 0.7
	treated	18.6 ± 2.5	13.2 ± 3.5	31.8 ± 5.6[g]	50.8 ± 0.3	59.0 ± 0.4
Cull Cows	control	–	36.1 ± 2.7	36.1 ± 2.7[fg]	–	60.0 ± 0.6
	treated	20.0 ± 1.5	20.0 ± 3.6	40.0 ± 4.9[ef]	49.5 ± 0.4	60.2 ± 0.4

[a] Incubation conditions are those described in Materials & Methods. In every case, the enzyme/substrate ratio was 1/100.
[b] qt: initial denaturation temperature.
[c] The shape of the curve did not allow for calculation qt (see fig. 1A).
[d–h] Values within columns followed by the same superscript letter do not differ significantly ($P < 0.05$).

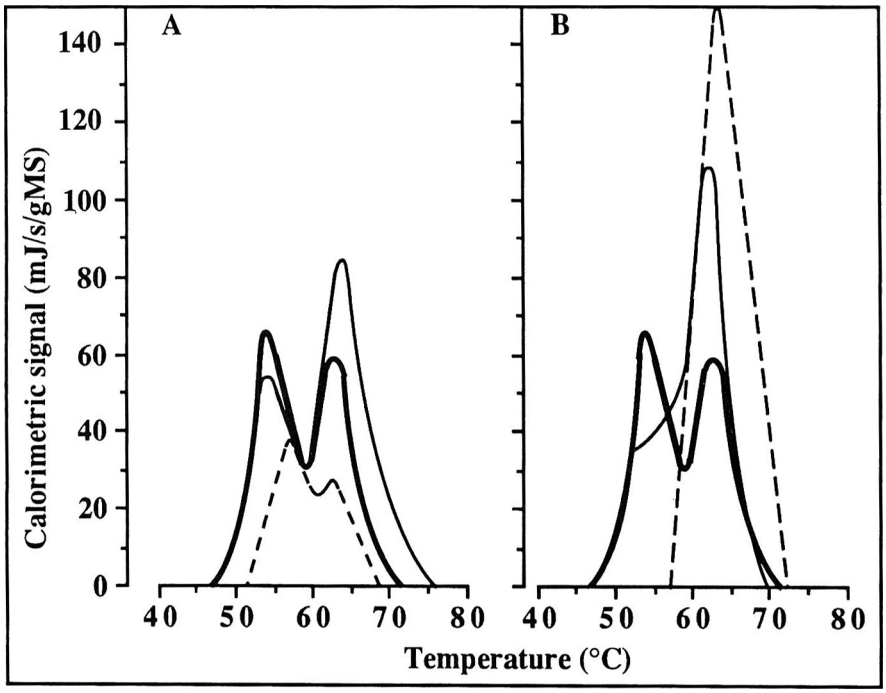

Fig. 1—Thermal denaturation curves of collagenase treated intramuscular collagen. (A) calf (– –), steer (——) and cull cow (——); (enzyme/substrate ratio 1/100). (B) untreated (– – –) and collagenase-treated steer collagen using an enzyme/substrate ratio 1/750 (——), and 1/100 (——); (Incubation conditions described in Materials & Methods section).

using indium and pure water as calorimetric standards. Quadruplicate collagen samples (10–20 mg) were covered with about 100 μL of either distilled water (results shown in Table 1) or 50 mM Tris-HCl buffer pH 7 containing 0.15M NaCl and 20 mM $CaCl_2$ (see Table 2) and sealed into stainless steel pans. After incubation for 24 hr at 30°C, treated and control samples were heated in the calorimeter from 25°C to 100°C at 3°C/min. A sealed pan containing an equivalent weight of Tris-HCl buffer was used as reference. As previously described (Kamoun et al., 1989), the thermodynamic parameters (enthalpy and temperatures of denaturation) were calculated from the thermograms recorded with a Sefram recorder using a custom program adapted to a Hewlett-Packard HP-85 Computer.

RESULTS & DISCUSSION

TABLE 1 shows the main characteristics of intramuscular collagen extracted from calves, steers and cull cows. Clearly a large decrease occurred in the thermal solubility of collagen with animal age thus confirming the well known close relationship between age and thermal solubility (Kopp and Bonnet, 1971). Indeed, intramuscular collagen from the oldest animals was much more tightly crosslinked and had higher thermostability. As already reported by Bernal and Stanley (1986), this change was strengthened by a concomitant increase in the initial temperature of denaturation. Calf collagen showed the lowest initial denaturation temperature followed by steer and cull cow collagen. Hence, we observed a close relationship ($r = 0.99$) between thermal solubility and initial temperature of denaturation. Therefore, note that together with the thermal solubility, determination of the initial temperature of denaturation might constitute a useful method for characterization of the crosslinking state of collagen. The greater susceptibility to heat denaturation of the less reticulate form of collagen (after collagenase treatment) could then serve as an indirect method to characterize the degree of reticulation of a collagen. Denaturation characteristics combined with thermal solubility are potential methods for investigating the degree of reticulation. A possible improvement over these usual methods could be the study of denaturation characteristics after histolyticum collagenase treatment.

Results reported in Tables 1 and 2 clearly showed that incubation conditions (24 hr at 30°C, pH 7.5) led to a decrease of 4–5°C in the initial temperature of collagen denaturation. This decrease was irrespective of the collagen crosslinking state. A partial increase in fragility of the structure by the long heating period at relatively low temperature, in the presence of calcium ions, might account for this enhancement in thermosensitivity of the different collagens.

Treatment with the collagenase from *Clostridium histolyticum* affected to a much greater extent the thermal stability of collagen (Table 2). This modification of the collagen thermostability allowed a quantification of the extent of collagen breakdown. It appeared to depend on age, greater changes being observed for younger animals. Collagenase treatment induced a significant decrease in the total enthalpy of denaturation (ΔH) of calf (77%) and, though to a lesser extent, of steer (25%) collagen whereas cull cow collagen did not change. These results were in good agreement with those obtained for insoluble endomysial and perimysial connective tissue by Tun-

ick (1988). Regarding the collagen from cull cows, its higher resistance to collagenase could have resulted from its greater degree of reticulation which led to a very low thermal solubility (Table 1).

The appearance of a new peak of denaturation with significantly lower initial temperature of denaturation [qt] (ranging from 49 to 54°C) was another obvious change due to collagenase treatment. The relative importance of these peaks, which showed maximum denaturation at 55–57°C (peak 1) and 62–63°C (peak 2), depended on both the crosslinking state of the collagen and the enzyme/substrate concentration ratio. As shown in Fig. 1A, the area ratio between peak 1 and peak 2 clearly decreased with age from 2.2 for calf collagen to 1 for cull cow collagen; steer collagen was intermediate with a ratio value of 1.4. This fact suggested a greater sensitivity to this proteinase of intramuscular collagen from younger animals, a result that agreed well with those of Bailey and Etherington (1985).

Intensity of the first peak showing a maximum of denaturation at about 55–57°C increased proportionally to the collagenase/collagen ratio (Fig. 1B). Using steer collagen, we tested two enzyme/substrate ratios (1/750 and 1/100). At the lowest enzyme concentration (E/S = 1/750), peak 1 appeared as a shoulder of the main peak of denaturation. Increasing the collagenase level (E/S = 1/100) led to the appearance of two well resolved peaks.

CONCLUSIONS

THERMAL STABILITY of collagen was markedly affected by treatment with a collagenase from *Clostridium histolyticum*. This proteinase induced a decrease in both the total enthalpy of denaturation ΔH and the initial temperature of denaturation [qt]. The extent of these changes was highly dependent on the crosslinking state of the substrate, the enzyme effect decreasing significantly as degree of reticulation of collagen increased. Moreover, this enzyme led to the appearance of a new peak of denaturation with a much lower initial temperature of denaturation. The size of the peak was closely dependent on both the crosslinking state of the collagen and the enzyme/substrate ratio. This DSC approach for investigating the action of a collagenase on collagens with different degrees of reticulation could thus serve as a reference method for comparative studies of the effects of other potential collagenolytic enzymes.

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Encapsulation of the Pre-Formed Cooked Cured-Meat Pigment

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ABSTRACT

The pre-formed cooked cured-meat pigment (CCMP) was stabilized by storage under a nitric oxide atmosphere or microencapsulation in food-grade carbohydrates. Amongst the wall materials tested, β-cyclodextrin, N-LOK, and Maltrin M-250 served as the best encapsulating agents. Presence of gum acacia or a mixture of sodium tripolyphosphate, sodium acid pyrophosphate and ascorbyl palmitate at a 5% level in the wall material(s) improved the color of meats treated with the powdered cooked cured-meat pigment (PCCMP). Some encapsulated pigments remained stable during an 18 month testing period under refrigeration. Spectral characteristics of PCCMP were similar to those of the freshly-prepared pigment and color stability of PCCMP-treated meats was similar to that of nitrite-cured and CCMP-treated products.

INTRODUCTION

ONE OF THE MOST important sensory characteristics of nitrite-cured meat products is their attractive color which is highly appealing to consumers and is generally equated with quality (Fox, 1966; MacDougall et al., 1975; Shahidi, 1989a, b). In addition, nitrite in the cure is responsible for development of a delicate flavor in meats by virtue of its antioxidant properties (Fooladi et al., 1979; Igene et al., 1985; Shahidi, 1989b; Shahidi et al., 1990) which may also be duplicated, to a certain degree, using natural and/or synthetic ingredients. Above all, the antimicrobial effect of nitrite is responsible for extended shelf-life of the cured products (Sofos et al., 1979; Pierson and Smoot, 1982).

Since nitrite is responsible for imparting several desired properties to cured meats, its replacement has been attempted (Sebranek, 1979) by developing combinations of various ingredients to provide different functions of nitrite (Sweet, 1975; Shahidi et al., 1988, 1990; Shahidi, 1989a; Shahidi and Pegg, 1991). However, these are not applicable to all types of products. Furthermore, it should be noted that a patent by Sweet (1975) presented the original idea of formulating composite nitrite-free meat curing systems consisting of a colorant, an antimicrobial agent, and an antioxidant/chelator. We used that approach in formulating nitrite-free compositions in which the colorant was the pre-formed cooked cured-meat pigment (CCMP).

An essential ingredient of the composite non-nitrite curing mixtures (Shahidi, 1989a; Shahidi et al., 1990) was the CCMP. Shahidi et al. (1984, 1985) prepared the pigment indirectly from bovine red blood cells through a hemin intermediate or directly from it (Shahidi and Pegg, 1988). The pre-formed CCMP successfully duplicated the characteristic color of nitrite-cured meats as analyzed by subjective (Shahidi et al., 1984; 1985; Pegg and Shahidi, 1987) or by objective (Shahidi and Pegg, 1988; 1990, 1991; Shahidi, 1989a; Shahidi et al., 1990) methods. Its application to emulsion meat products in preparation of wieners has also been successful (Pegg and Shahidi, 1987; Shahidi et al., 1990; O'Boyle et al., 1990). Thus, CCMP when used in conjunction with other ingredients, presented a means of entirely eliminating nitrite from meat curing practice.

Due to the sensitivity of the CCMP to light and oxygen (Shahidi et al., 1985) its stabilization and protection is highly desirable. We designed the present study to investigate two novel approaches for enhancing the storage stability of the pre-formed CCMP and to examine the color characteristics of pigment-treated meat products.

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MATERIALS & METHODS

Materials

All chemicals were reagent- or food-grade materials, unless otherwise specified, and were used without further purification. Hemin was prepared by deproteinization of bovine red blood cells (Shahidi et al., 1984) or was purchased from Sigma Chemical Company (St. Louis, MO). β-cyclodextrin, Ringdex-B®, was provided by Toyomenka (America Inc.), New York. N-LOK was acquired from National Starch and Chemical Corporation (Bridgewater, NJ). Modified (etherified) β-cyclodextrin lot RR3-HE Series was obtained from American Maize-Products Company (Hammond, IN). All Maltrin samples were supplied by the Grain Processing Corporation (Muscatine, IA). Sodium tripolyphosphate (STPP) and sodium acid pyrophosphate (SAPP) were obtained from Albright and Wilson Americas (Toronto, Ontario). Ascorbic acid (AA) and ascorbyl palmitate (AP) were supplied by Hoffmann-La Roche Limited (Toronto, Ontario). Gum acacia was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI).

Preparation of the cooked cured-meat pigment (CCMP)

The cooked cured-meat pigment was prepared from hemin and nitric oxide (Shahidi et al., 1985) or directly from bovine red blood cells as described (Shahidi and Pegg, 1988; 1990b). A typical medium scale preparation method of the CCMP from hemin is outlined below:

Bovine hemin (600 mg) was dissolved in 100 mL of a 0.04 M Na_2CO_3 solution. While in the dark and under a nitrogen atmosphere, 10.0g sodium dithionite or sodium ascorbate with or without 10.0g sodium tripolyphosphate were added to the mixture. At this stage either 500 mL more Na_2CO_3 solution or 900 mL of a 0.2M acetate buffer (pH = 6.5) was added. Nitric oxide was then slowly bubbled into the mixture for about 5 min. The container was then capped and stored in the dark until use. The pre-formed cooked cured-meat pigment was recovered as a precipitate from the mixture after its centrifugation and repeated washing with a 2% (w/v) ascorbic acid solution to ensure elimination of any traces of nitrite from the mixture.

Preparation of the powdered cooked cured-meat pigment (PCCMP)

A Büchi Mini Spray Dryer (Model 190), Büchi Laboratory-Techniques Limited, Flawil, Switzerland was used for preparation of the PCCMP. Nitrogen was used as the spray flow gas (O'Boyle, 1986) to minimize contact between the preformed pigment and oxygen. Optimized spray drying conditions were: inlet ≥ 150°C; outlet 98°C; feed flow 5.5 mL·min^{-1} and nitrogen pressure 375 kPa(g).

An emulsion of the pigment and encapsulating agent(s) was formulated prior to spray drying. The encapsulating agents (or wall materials) investigated were N-LOK, β-cyclodextrin, modified β-cyclodextrin, gum acacia and Maltrin M-250. Wall materials, used individually or in combination, were generally employed at a 1.5% (w/w) final level [i.e., the ratio of the pigment to wall material(s)]. STPP, SAPP, and AP were used in combination with some of the

above encapsulating agents. The optimum level was selected based on examination of Hunter L, a, b value of a typical set of PCCMP-treated samples.

To prepare the emulsion, the wall material(s) were first dissolved or dispersed in water. Addition of a few drops of sodium hydroxide helped to increase the solubility of β-cyclodextrin and some other wall materials. The pigment was then introduced into this mixture together with AA at a CCMP/AA ratio 1:2 (w/w). The emulsion was diluted with water generally to 3.5% (w/w) and in some cases up to 10.0% (w/w) solids. Higher solids, at conditions employed, did not allow dissolution of CCMP in the mixtures. This solution was thoroughly stirred to ensure uniform dispersion of the pigment. The vessel containing the emulsion was covered with parafilm and aluminum foil to minimize exposure of pigment to oxygen and light. The emulsion was then spray dried at the operating conditions stated earlier, unless otherwise specified.

Preservation of CCMP under nitric oxide and in dark

The pigment precipitates (100–1000 mg) were washed with 3 × 20 mL of a 2% (w/v) AA solution and were then transferred into an amber-colored ampule. After centrifugation, the supernatant was discarded and the precipitates were covered with a 20–50 mL supersaturated nitric oxide solution containing 2% (w/w) AA. The ampule was then frozen in liquid nitrogen and sealed with a flame. Sealed pigment tubes were opened after 3, 6 and 9 mo storage. The quality of the pigment was checked by monitoring its absorbance at 540 and 563 nm. The absorption intensities at these wavelengths and relative changes were noted. Pigment stored for 9 mo was also applied to meat to check coloring potency and quality.

Application of CCMP to comminuted meats

Fresh pork loin, from Newfoundland Farm Products (St. John's, Newfoundland), was trimmed of most exterior fat and ground twice with a 0.79 cm and then a 0.48 cm plate. Ground pork was mixed with 20% (w/w) distilled water and 550 ppm sodium ascorbate. The pigment preserved in a nitric oxide solution in sealed amber-colored ampules or the PCCMP from different formulations was added to meat samples at levels of 12 or 40, 50 and 60 ppm. The systems were mixed thoroughly to obtain homogenized samples. Meats were then cooked either in a glass container or in casings in moulded metal containers (15 cm × 1.8 cm, i.d.) at 85 ± 2°C in a thermostat-controlled water bath for about 40 min to reach an internal temperature of 75 ± 2°C, while stirring occasionally with a glass rod. After cooling to room temperature, cooked meat samples were homogenized in a Waring blender for 30 sec and then stored in 4.5 mm thick Kapak/Scotchpak heat sealable pouches (Stock No. 502).

Elimination of unbound nitrite from pre-formed CCMP

The precipitated pigment was separated after decanting the supernatant. This pigment was then applied to ground meat at 12 ppm addition. The color of the cooked treated-meat was evaluated by its Hunter L, a, b parameters. In another set of experiments three tubes containing the precipitated pigment were mixed with 2 mL of a 2% (w/v) AA solution. The mixture was vortexed for 20 sec. The tubes were then centrifuged and the supernatant was decanted. One pigment tube was retained for subsequent addition to meat. The washing procedure was repeated. The pigments obtained after a second and a third wash were also applied to ground meat and the color was evaluated on the resultant samples. Finally, a pigment sample which was washed twice with the AA solution was added at 12 ppm to a meat formulation. A nitrite-cured control was also used. Color characteristics after cooking were then examined.

Analyses of pigment-treated meats

The pigment from hemin-nitric oxide synthesis or produced directly from bovine red blood cells, as well as the stored pigment in a nitric oxide solution or in the powdered form was dissolved or extracted in 4:1 (v/v) acetone/water solutions. The samples were then analyzed by the method of Hornsey (1956) and their absorption spectra recorded using either a Shimadzu UV-260 or a Beckman DU-8 spectrophotometer. Acetone/water extracts of PCCMP were filtered through Whatman No. 3 filter paper before spectral analysis to avoid problems of turbidity arising from interference of insoluble wall materials. Meat pigments from nitrite-cured and pigment-treated pork, after cooking, were extracted into 4:1 (v/v) acetone/water (Hornsey, 1956). All absorption spectra in the visible range were recorded. A Model XL-20 Tristimulus Colorimeter, Gardner Laboratory Inc., was used to determine lightness/darkness (L value), red/green (+/− a value), and yellow/blue (+/− b value) of treated meat samples. A white ceramic tile, provided by Gardner Laboratory Inc., with specifications L = 92.0, a = −1.1, and b = 0.7 was used to standardize the colorimeter. Tristimulus color values (L, a, b) were measured at 3–5 different locations on the meat surface. Generally 3 replicates with the same original meat sample were made.

Color stability of the treated meat samples

Comminuted meat samples treated with one of the following: 156 ppm sodium nitrite, 12 ppm freshly prepared CCMP, 12 ppm pigment stored for 9 mo under nitric oxide, and 50 ppm of the PCCMP encapsulated in selected wall materials, were subjected to intense fluorescent lighting. Packaged meat samples were placed 25 cm under a set of two 30-Watt fluorescent "Daylite" lamps in a 4°C walk-in refrigerator. Samples were withdrawn after different storage times for color evaluation.

Statistical analyses

All experiments and/or measurements were replicated 3 to 6 times. Means ± standard deviations were recorded in each case. Analysis of variance and Tukey's studentized range tests (Snedecor and Cochran, 1980) were used to determine differences in mean values. Significance was determined at $P \geq 0.05$.

RESULTS & DISCUSSION

ALTHOUGH a large number of experiments were performed and many different wall material combinations or encapsulat-

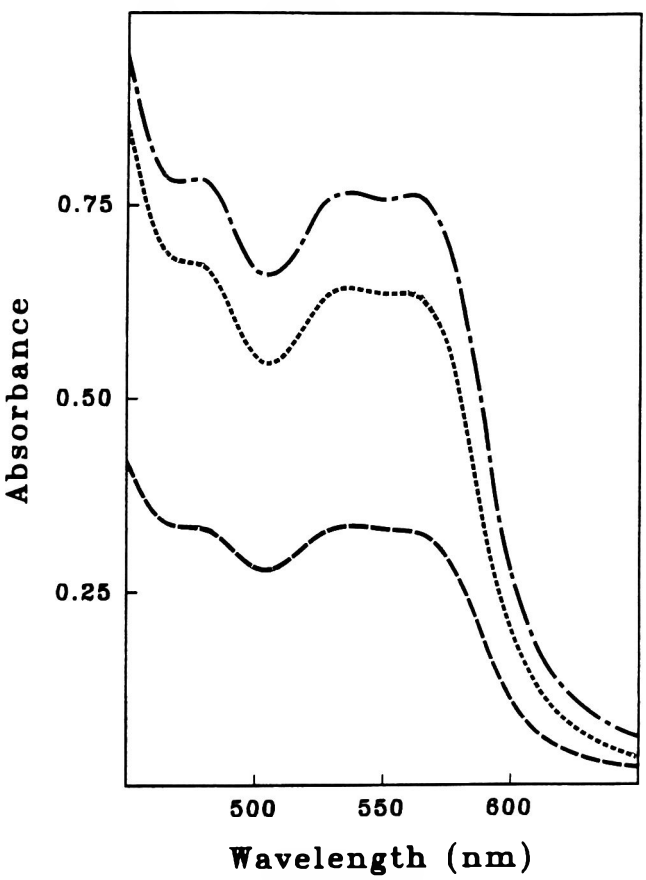

Fig. 1—Absorption spectra of: powdered cooked-cured meat pigment (PCCMP) with N-LOK wall material, •——•; cooked cured-meat pigment (CCMP) from hemin/nitric oxide synthesis, ••••••••; and pigments extracted from nitrite-cured ham, •— — — — —. All pigments were dissolved/extracted in acetone/water (4:1, v/v).

Table 1—Effect of final level on Hunter L, a, b values of meats cooked with powdered cooked cured-meat pigment (PCCMP)[y]

Exp no.	Treatment	Wall materials	Payload %	Hunter values[z] L	a	b
1	No additive	–	–	59.0 ± 0.2[a]	4.7 ± 0.2[d]	11.4 ± 0.1[a]
2	$NaNO_2$, 156 ppm	–	–	58.4 ± 0.1[b]	11.8 ± 0.2[bc]	9.1 ± 0.1[b]
3	CCMP, 12 ppm	–	–	57.9 ± 0.2[b]	11.7 ± 0.2[bc]	9.1 ± 0.1[b]
4	PCCMP, 50 ppm	95% N-LOK	0.67	52.0 ± 0.2[d]	11.8 ± 0.2[bc]	9.1 ± 0.1[b]
5	(4)	(4)	1.0	52.1 ± 0.2[d]	11.9 ± 0.2[b]	9.1 ± 0.1[b]
6	(4)	(4)	1.5	52.5 ± 0.1[d]	12.9 ± 0.1[a]	8.9 ± 0.1[c]
7	(4)	(4)	2.0	53.8 ± 0.2[c]	11.3 ± 0.2[c]	8.7 ± 0.2[c]
8	(4)	(4)	3.0	53.6 ± 0.2[c]	11.3 ± 0.1[c]	8.7 ± 0.2[c]

[a-d] Mean values of 3 to 6 replicates ± standard deviations. Values with same superscripts not significantly (P > 0.05) different.
[y] Percent solids was 10%.
[z] Wall materials contained 2% STPP, 2% SAPP and 1% AP.

Table 2—Effect of inlet temperature of spray dryer on the Hunter L, a, b values of meats cooked with powdered cooked cured-meat pigment[y]

Exp no.	Inlet temp °C	Hunter values L	a	b
1	130	52.7 ± 0.2[a]	11.8 ± 0.1[b]	9.1 ± 0.2[a]
2	150	52.5 ± 0.1[a]	12.9 ± 0.1[a]	8.9 ± 0.2[a]
3	150[z]	52.8 ± 0.2[a]	12.6 ± 0.2[a]	9.0 ± 0.2[a]
4	170	52.4 ± 0.1[a]	12.6 ± 0.2[a]	9.2 ± 0.2[a]

[a-b] Mean values of 3 replicates ± standard deviation. Values with same superscripts not significantly (P > 0.05) different.
[y] Wall materials were 95% N-LOK, 2% STPP, 2% SAPP and 1% AP. PCCMP added at 50 ppm, unless otherwise specified.
[z] PCCMP added at 35 ppm.

Table 3—Effect of pigment concentration on Hunter L, a. b values of cooked meats[z]

Exp no.	treatment	Hunter values L	a	b
1	No additive	59.0 ± 0.2[a]	4.7 ± 0.1[d]	11.4 ± 0.1[a]
2	$NaNO_2$, 156 ppm	58.4 ± 0.1[b]	11.8 ± 0.2[c]	9.1 ± 0.1[b]
3	CCMP, 12 ppm	57.9 ± 0.2[c]	11.7 ± 0.2[c]	9.1 ± 0.1[b]
4	PCCMP, 30 ppm	54.5 ± 0.2[d]	12.2 ± 0.2[bc]	9.1 ± 0.1[b]
5	PCCMP, 50 ppm	52.5 ± 0.1[e]	12.9 ± 0.1[a]	8.8 ± 0.1[c]

[a-e] Mean values of 6 replicates ± standard deviations. Values with same superscripts not significantly (P > 0.05) different.
[z] A final level of 1.5% (w/w) was used. Wall materials for PCCMP were N-LOK, 95%; STPP, 2%; SAPP, 2%; and AP, 1%. All samples included 550 ppm sodium ascorbate.

ing agents were tested, only some representative results are shown here. In all cases reported, the color quality of meats treated with the PCCMP was generally compared with those treated with 12 ppm of freshly prepared CCMP and 156 ppm of sodium nitrite. We have previously shown color of meats treated with 12 ppm CCMP to be indistinguishable from that of nitrite-cured counterparts (Shahidi and Pegg, 1990). Although examination of color by sensory means might be preferred, we have found that it did not offer any further information in our study (Shahidi and Pegg, 1988). Thus, color parameters of treated meats which closely resembled those of nitrite cured counterparts (± 1 Hunter a value) were considered desirable. Although smaller differences observed by instrumental means were statistically significant ($P<0.05$), these were not easily noticeable as judged by experimenters who were most familiar with these systems. Furthermore, such samples were generally found to be indistinguishable from those of nitrite-cured counterparts. Treated samples which did not satisfy this condition were always found to be undesirable and visually different from those cured with nitrite.

Figure 1 compares the typical absorption pattern, in the visible region, of a PCCMP sample in acetone/water (Hornsey, 1956) with that of freshly prepared CCMP or that extracted from a nitrite-cured meat sample. All pigment solutions showed the characteristic absorption pattern of iron-porphyrin compounds with a red color and had maxima at 540 and 563 nm. Furthermore, pigments extracted from cooked PCCMP-treated meats exhibited an identical absorption pattern and maxima to those reported above. Thus, it might be reasonable to assume that microencapsulation and spray drying did not alter the chemical nature of the CCMP. However, this was not verified. The most important variables in preparation of PCCMP were type of wall materials used as well as the final level and the inlet temperature of the spray dryer. Other parameters such as feed flow rate and nitrogen pressure were less important.

Hunter L,a,b values of a typical set of PCCMP-treated meat samples were monitored in order to set the optimum final level of the pigment in encapsulating materials. The best encapsulated pigments had a final level of 1 to 1.5% (Table 1). Furthermore, as the final level was increased from 1 to 1.5%, a significant ($P<0.05$) increase in Hunter a value of meats treated with PCCMP was noted. However, at higher levels, less wall material was available to protect the preformed CCMP. In those, the effective color imparted by the spray-dried pigment was significantly reduced ($P<0.05$). This was shown by a decrease in Hunter a values (Table 1). In all experiments related to pink color intensity of products, a higher Hunter a value was preferred since it potentially allowed less PCCMP addition to meats to achieve a given final color.

Of the several variables in the spray-drying condition of CCMP, inlet temperature was found to be of critical importance. Typical results (Table 2) indicated than an inlet of ≥ 150°C afforded the best quality PCCMP as judged by Hunter color measurement of the PCCMP-treated meats. As the inlet temperature was increased from 130 to 150°C, a significant ($P<0.05$) increase in Hunter a value of the PCCMP-treated meat was noticeable. Variations of the inlet temperature did not affect Hunter L or b values of treated meats. Since the spray performance depended on the inlet temperature, at a given flow and aspiration rate, a temperature of 150–170°C could be used. We selected an inlet temperature of 150°C for other experiments.

The effect of concentration of PCCMP on color intensity of treated meats was also monitored. Table 3 summarizes typical results for PCCMP-treated meats in which combinations of wall materials consisted of 95% N-LOK, 2% STPP, 2% SAPP and 1% AP. Results indicated that PCCMP-treated samples at 30-40 ppm levels resembled most the color of nitrite-cured meats. Higher addition levels of PCCMP significantly ($P<0.05$) increased Hunter a values and decreased Hunter L values of treated samples. However, the effect may not be visually unattractive. Nonetheless, the optimal addition level of spray-dried pigment to meat depends primarily on its original myoglobin content (Shahidi and Pegg, 1988; 1991) as well as the conditions under which encapsulation was performed.

Based on the above experiments and under the best conditions specified above, the color characteristic of PCCMP-treated meats was tested as a function of different wall material(s). Typical results of the study are provided in Table 4. Generally carbohydrates are used in microencapsulation processes because of their low cost and good functionality (Reineccius, 1990). Simple starch hydrolyzates, modified starches or various gums are used. In our work, of the wall materials examined individually, N-LOK, β-cyclodextrin and Maltrin M-

Table 4—Hunter L, a, b values of PCCMP-treated meats as affected by wall material(s)[z]

Exp no.	Additives	Wall material(s)	Hunter values[z] L	a	b
1	No additive	–	59.0±0.2[a]	4.7±0.1[k]	11.4±0.1[a]
2	$NaNO_2$, 156 ppm	–	58.4±0.1[ab]	11.8±0.2[ef]	9.1±0.1[d]
3	CCMP, 12 ppm	–	57.9±0.2[b]	11.7±0.2[efg]	9.1±0.1[d]
4	PCCMP, 50 ppm	N-LOK	54.0±0.2[ghi]	11.9±0.2[de]	9.2±0.1[cd]
5	(4)	β-cyclodextrin	54.7±0.1[cdef]	11.7±0.1[efg]	9.1±0.1[d]
6	(4)	Modified β-cyclodextrin	54.0±0.2[ghi]	9.0±0.1[d]	9.6±0.1[bc]
7	(4)	Maltrin M-250	53.3±0.2[jkl]	11.6±0.1[efg]	9.7±0.2[b]
8	(4)	Gum Acacia	53.4±0.2[ijkl]	11.1±0.2[h]	9.2±0.2[cd]
9	(4)	95% (4) + 5% (8)	53.2±0.2[kl]	12.4±0.2[bc]	9.1±0.1[d]
10	(4)	95% (4) + 5% (COMBO)	52.8±0.2[l]	12.9±0.1[a]	9.1±0.1[d]
11	(4)	90% (4) + 5% (8) + 5% (COMBO)	54.8±0.2[cde]	11.1±0.1[h]	9.3±0.2[bcd]
12	(4)	85% (4) + 15% (5)	53.9±0.2[ghij]	11.3±0.1[gh]	9.2±0.1[cd]
13	(4)	80% (4) + 15% (5) + 5% (8)	54.0±0.2[ghi]	11.7±0.1[efg]	9.2±0.1[cd]
14	(4)	80% (4) + 15% (6) + 5% (8)	53.6±0.1[hijk]	11.4±0.1[fgh]	9.2±0.1[cd]
15	(4)	80% (4) + 15% (5) + 5% (COMBO)	53.2±0.2[kl]	12.6±0.1[ab]	9.0±0.2[d]
16	(4)	75% (4) + 15% (5) + 5% (8) + 5% (COMBO)	53.3±0.2[jkl]	12.0±0.1[cde]	8.9±0.1[d]
17	(4)	70% (4) + 30% (5)	53.2±0.2[kl]	11.9±0.2[de]	9.1±0.1[d]
18	(4)	98% (5) + 1% (STPP) + 1% (SAPP)	55.1±0.2[c]	10.5±0.1[i]	9.3±0.2[bcd]
19	(4)	95% (5) + 5% (COMBO)	53.4±0.2[ijkl]	11.4±0.2[fgh]	9.1±0.1[d]
20	(4)	95% (5) + 5% (8)	53.3±0.1[jkl]	12.3±0.1[bcd]	9.3±0.1[bcd]
21	(4)	90% (5) + 5% (8) + 5% (COMBO)	54.9±0.2[cd]	11.1±0.2[h]	9.6±0.1[bc]
22	(4)	95% (6) + 5% (COMBO)	54.0±0.1[ghi]	9.1±0.1[i]	9.2±0.2[cd]
23	(4)	95% (7) + 5% (COMBO)	54.4±0.2[defg]	12.3±0.1[bcd]	9.2±0.2[cd]
24	(4)	80% (7) + 15% (5) + 5% (COMBO)	54.1±0.1[fgh]	11.9±0.1[de]	9.2±0.1[cd]
25	(4)	80% (7) + 15% (6) + 5% (COMBO)	54.2±0.5[efgh]	11.0±0.1[h]	9.3±0.1[bcd]

[a-l] Mean values of 3 replicates ± standard deviation. Values with same superscripts not significantly (P>0.05) different.

[z] All samples cooked with 20% water and contained 550 ppm sodium ascorbate. COMBO = combination of STPP/SAPP/AP (2:2:1, w/w/w). An average yield of 59–68% (max 76%) of encapsulated product was obtained in all cases. Samples kept refrigerated (2–4°C).

250 most closely resembled the color characteristics of nitrite-cured meat which were also indistinguishable from that of meat treated with 12 ppm of freshly prepared CCMP. In Maltrin Series, in addition to Maltrin M-250, other grades of Maltrin namely M-040, M-100, M-150, M-200, M-500 and M-700 were tested. Only marginal differences were apparent with different Maltrins (results not shown). Modified β-cyclodextrin (etherified) with obscured hydroxyl groups (Parmerter et al., 1969) and gum acacia, however, were less effective in protection of CCMP (Table 4).

Protection of the CCMP by β-cyclodextrin may arise from full or partial inclusion of CCMP in its central cavity or simply by it becoming surrounded with β-cyclodextrin. In general to form inclusion compounds, the material to be encapsulated is added to a warm aqueous solution of β-cyclodextrin. Equilibrium is reached with intense stirring. Under slow cooling, the inclusion complex precipitates and afterwards is recovered by filtration. On the other hand, water may be removed from the system by freeze- or spray-drying (Szejtli, 1982). In our studies, however, CCMP was added to a basic solution of β-cyclodextrin at room temperature. Since precipitation of the mixture under these conditions is unlikely (Szejtli, 1982), dehydration by spray drying was used. Furthermore, sensitivity of CCMP necessitated a short-time handling to retard possible decomposition. Despite excellent encapsulating ability for different applications (Yamada et al., 1980; Pitha, 1981; Szejtli, 1981; Shaw et al., 1984; Martin et al., 1990), β-cyclodextrin is not yet permitted as a food ingredient in many countries.

In another set of experiments, several combinations of the above wall materials were employed. The major surrounding wall material was N-LOK, β-cyclodextrin, modified β-cyclodextrin, or Maltrin M-250. Generally addition of gum acacia to combinations containing the above wall materials improved their performance as was noticeable in the Hunter a values of PCCMP-treated meats (Table 4). Furthermore, in all cases examined, addition of a 5% mixture of STPP/SAPP/AP (2:2:1, w/w/w) to the wall material(s) improved performance of the encapsulated pigment. Larger Hunter a values were evident where this mixture was present (Table 4). A similar visual color effect was apparent when polyphosphates were added to CCMP-treated meats (Shahidi and Pegg, 1990a).

The effect of washing of the CCMP with an AA solution prior to encapsulation, to remove any unbound nitrite, on Hunter L, a, b values of treated meats was studied. Results indicated that presence of any residual nitrite enhanced the color effect of the pigment, thus leading to higher Hunter a values. Therefore, a decrease in Hunter a values of treated meats from 11.7±0.2 to 11.3±0.1, 11.1±0.1 and 11.0±0.2, after sequential washings with AA solution was observed. Encapsulation of unwashed pigment and subsequent application to meat gave a Hunter a value 12.0±0.1 after cooking and that of washed pigment afforded a value of 10.8±0.2.

Stability of the CCMP stored up to 9 mo in amber-colored ampules and under a positive pressure of nitric oxide was tested by examining its absorption maxima at 540 and 563 nm (Hornsey, 1956). The absorbance values of these pigments did not change significantly (P>0.05) from those of freshly prepared counterparts over the test period (results not shown). After the 9 mo storage, the pigment was applied to ground pork. A control sample using freshly prepared CCMP was used for comparison. The color quality of the samples, as judged by Hunter L, a, b values, changed from 57.8±0.1, 11.8±0.1 and 9.2±0.1 (control) to 57.5±0.1, 11.5±0.1 and 9.1±0.2. While Hunter b values were not significantly (P>0.05) different from one another, Hunter L and a values were marginally different from those of the meat cooked with a fresh sample of CCMP.

The effect of storage of encapsulated pigment, PCCMP, and its performance was monitored. Hunter a values of treated meats as a function of storage time generally indicated that the coloring quality of PCCMP was primarily dictated by its initial color properties (Table 5). Therefore, the color of samples encapsulated with modified β-cyclodextrin remained less desirable as their Hunter a values were more than one unit below those of nitrite-cured counterparts. Samples containing STPP/SAPP/AP combinations or gum acacia had more desirable coloring properties, thus indicating that protected pigments retained their structural integrity. However, note that when the pigments were stored at room temperature, their stability was adversely affected (results not shown). Therefore, we suggest that the encapsulated pigments be preferably stored at refrigeration temperatures to enhance shelf-life.

Finally, the effect of intense fluorescent lighting on color stability of meats treated with PCCMP was examined. Typical results using encapsulating materials with different protection characteristics, as judged by initial Hunter a value of freshly

Table 5—Effect of storage on Hunter L, a, b values of meats cooked with powdered cooked cured-meat pigment[z]

Exp no.	Wall material(s)	Storage time (months)	Hunter values L	a	b
1	N-LOK	0	54.0 ± 0.2^{mn}	11.9 ± 0.2^{bcd}	9.2 ± 0.1^{ef}
		9	57.2 ± 0.1^{ab}	10.9 ± 0.2^{g}	9.7 ± 0.2^{abcd}
2	N-LOK (95%) + COMBO (5%)	0	52.8 ± 0.2^{p}	12.9 ± 0.1^{a}	9.1 ± 0.1^{f}
		2.5	55.2 ± 0.1^{ghij}	12.1 ± 0.2^{bd}	9.4 ± 0.2^{cdef}
		9	55.7 ± 0.1^{efgh}	12.0 ± 0.2^{bcd}	9.4 ± 0.2^{cdef}
		18	55.8 ± 0.1^{efg}	11.7 ± 0.2^{cde}	9.2 ± 0.2^{ef}
3	N-LOK (95%) + Gum Acacia (5%)	0	53.2 ± 0.2^{p}	12.4 ± 0.2^{ab}	9.1 ± 0.2^{f}
		2.5	53.2 ± 0.1^{p}	12.3 ± 0.1^{b}	9.2 ± 0.2^{ef}
		9	53.4 ± 0.2^{nop}	12.3 ± 0.2^{b}	9.2 ± 0.2^{ef}
4	β-cyclodextrin	0	54.7 ± 0.1^{jkl}	11.7 ± 0.1^{cde}	9.1 ± 0.1^{f}
		11	55.3 ± 0.2^{ghij}	11.2 ± 0.2^{efg}	9.2 ± 0.1^{ef}
5	β-cyclodextrin (95%) + COMBO (5%)	0	55.1 ± 0.2^{hij}	11.5 ± 0.1^{def}	9.3 ± 0.1^{def}
		4	56.5 ± 0.2^{cd}	11.0 ± 0.2^{fg}	9.8 ± 0.1^{abc}
		18	56.2 ± 0.2^{def}	11.0 ± 0.2^{fg}	9.7 ± 0.2^{abcd}
6	β-cyclodextrin (95%) + Gum Acacia (5%)	0	55.0 ± 0.1^{ijk}	11.6 ± 0.2^{cde}	9.4 ± 0.1^{cdef}
		4	56.3 ± 0.2^{de}	11.2 ± 0.3^{efg}	9.6 ± 0.1^{abcde}
7	Modified β-cyclodextrin	0	54.0 ± 0.5^{mn}	9.0 ± 0.1^{i}	9.6 ± 0.1^{abcde}
		4	54.9 ± 0.3^{jk}	8.6 ± 0.2^{i}	9.7 ± 0.2^{abcd}
8	Maltrin M-250	0	53.3 ± 0.2^{op}	11 ± 0.1^{cde}	9.7 ± 0.2^{abcd}
		4	55.6 ± 0.1^{fgji}	10 ± 0.2^{g}	9.9 ± 0.1^{abc}
9	Maltrin (95%) + COMBO (5%)	0	54.4 ± 0.2^{klm}	12 ± 0.1^{b}	9.2 ± 0.2^{ef}
		11	56.0 ± 0.1^{def}	11 ± 0.3^{efg}	9.7 ± 0.2^{abcd}
10	N-LOK (85%) + β-cyclodextrin (15%)	0	53.9 ± 0.1^{mno}	11 ± 0.1^{fg}	9.5 ± 0.2^{bcdef}
		9	55.6 ± 0.1^{fghi}	10 ± 0.1^{h}	9.6 ± 0.1^{abcde}
11	Maltrin (85%) + β-cyclodextrin (15%)	0	54.1 ± 0.1^{lm}	11 ± 0.1^{bcd}	9.2 ± 0.1^{ef}
		11	56.0 ± 0.1^{def}	11 ± 0.2^{efg}	9.7 ± 0.1^{abcd}
12	(10) (95%) + COMBO (5%)	0	54.2 ± 0.5^{lm}	11 ± 0.1^{cde}	9.3 ± 0.1^{def}
		11	57.3 ± 0.1^{ab}	11 ± 0.3^{efg}	9.7 ± 0.1^{abcd}
13	Gum Acacia	0	57.0 ± 0.3^{bc}	8 ± 0.1^{l}	9.9 ± 0.2^{abc}
		4	57.7 ± 0.3^{a}	8 ± 0.1^{i}	10.0 ± 0.1^{a}

[a-p] Mean values of 3 replicates ± standard deviation. Values with same superscripts not significantly (P>0.05) different.
[z] All samples cooked with 50 ppm PCCMP. COMBO = combination of STPP/SAPP/AP (2:2:1, w/w/w).

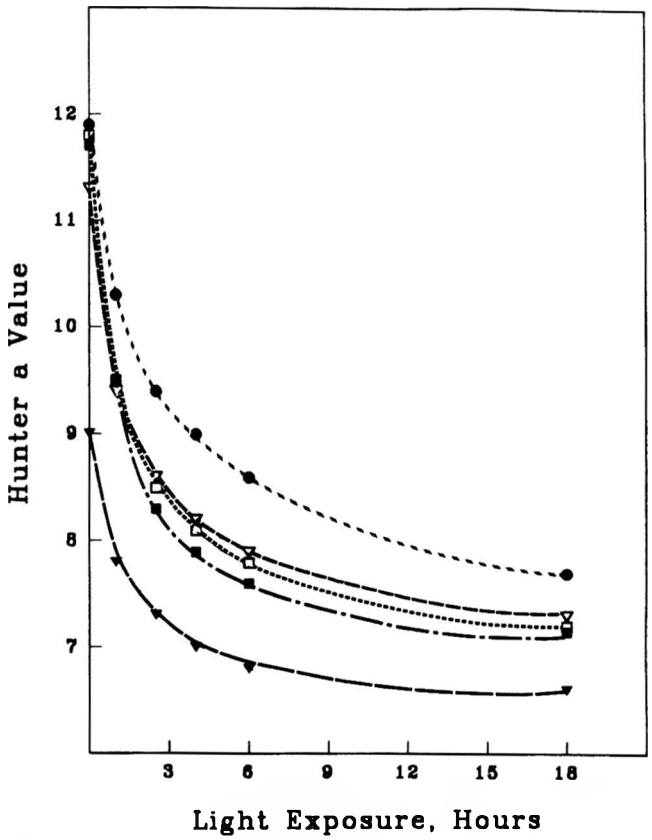

Fig. 2—Hunter a values of nitrite-cured, ——; and powdered cooked cured-meat pigment (PCCMP) treated meats, stored under fluorescent lighting at 4°C. Wall materials of PCCMP were: N-LOK, - - - -; N-LOK (85%) + β-Cyclodextrin (15%), – – – –; N-LOK (80%) + β-Cyclodextrin (15%) + gum acacia (5%), —·—; and modified-β-Cyclodextrin, ——.

encapsulated pigments, are shown in Fig. 2. In all cases, a drastic reduction in Hunter a values was evident during the first 6 hr of fluorescent lighting. The ultimate Hunter a value, after an 18 hr exposure was near that observed for meats cured with 156 ppm sodium nitrite (±1 Hunter a value). Nonetheless, this value depended, to some extent, on the initial color values of the treated meat samples and also on the total concentration of pigments in the muscle tissue homogenates (Fig. 2).

CONCLUSIONS

THE CCMP may be stabilized effectively by either storing it under a positive pressure of nitrite oxide or by its encapsulation in food-grade starch-based wall materials. Although stabilized pigments prepared by both methods had similar color qualities, the PCCMP may be more practical for use by processors. The color stability of the treated meat products was similar to their nitrite-cured analog. Thus, the presence of residual nitrite used in traditional curing of meat may not play an important role in color stability under extreme conditions.

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Efficacy of Pork Loin as a Source of Zinc and Effect of Added Cysteine on Zinc Bioavailability

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ABSTRACT

Based upon Zn uptake in chick tibia, Zn in raw and cooked pork loin was about 40% more bioavailable than that in $ZnSO_4 \cdot H_2O$. Neither roasting nor braising affected Zn bioavailability in the loin preparations. Adding 0.40% cysteine to diets containing $ZnSO_4$ improved Zn bioavailability by 73%. Pork loin is an excellent source of bioavailable Zn, and SH-containing compounds such as cysteine and glutathione that are present in meat may contribute to enhanced gut absorption of meat-source Zn.

Key Words: pork, zinc, bioavailability, cysteine chick-uptake,

INTRODUCTION

BIOAVAILABILITY OF ZINC in diets can be decreased by many factors, including high calcium intakes, low protein intakes and presence of phytic acid (O'Dell, 1969; Underwood, 1977; Solomons and Cousins, 1984). Zn absorption is affected by the level of Zn in the diet, low levels being absorbed more efficiently than high levels. Fruits and vegetables have a low Zn concentration. In the United States marginal Zn deficiency is of growing concern due to a low intake of foods high in Zn and the inhibitory effects of other dietary factors on Zn bioavailability (Welsh and Marston, 1983; Moser-Veillon, 1990).

The bioavailability of Zn from animal foods has been shown to be greater than that from plant food sources (O'Dell et al., 1972). The lower availability of Zn from plant sources is believed to be due to phytic acid and the formation of insoluble phytic acid-mineral complexes (Erdman and Forbes, 1981; Baker and Halpin, 1988). It has been postulated that the increased availability of Zn from animal (meat) sources is caused by a "meat factor" which is yet to be elucidated (Underwood, 1977; Solomons, 1982).

Because pork is an important source of Zn in the human diet (Welsh and Marston, 1983; Marchello et al., 1985), it deserves attention. Very little conclusive work has been done to determine Zn bioavailability from pork exposed to various conventional cooking methods. Although Zn bioavailability in beef is known to be very high (Brown et al., 1985; Gallaher et al., 1988), its bioavailability in pork has not been clearly established.

Addition of certain amino acids to diets may increase Zn absorption (Wapnir and Stiel, 1986). Because sulfhydryl compounds (eg., cysteine and glutathione) are high in meats and are known to bind trace elements and increase their absorption efficiency (Layrisse et al., 1984; Taylor et al., 1986; Wapnir and Stiel, 1986), it is possible that cysteine in either free form or as a peptide could be part of the elusive "meat factor" that is responsible for enhancing absorption of trace elements such as Zn and iron. Our objective was therefore, to determine Zn bioavailability in pork loin relative to $ZnSO_4$, and further, to ascertain whether adding an excess level of cysteine to the diet would increase the bioavailability of Zn from the $ZnSO_4$ standard.

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MATERIALS & METHODS

Loin preparation

Pork loins for both experiments were obtained from York-Duroc crossbred pigs bred and raised at the University of Illinois Swine Research Center. Center sections of loins were cut, trimmed to remove all but about 1 mm of subcutaneous fat, vacuum packaged (3 loins/pkg) and then stored frozen at −34° C. Prior to cooking, the loins were placed at 4° C overnight to thaw. Loins were cut into thirds after which they were allotted to three different treatments (no cooking, roasting, and braising). Hence, the center and tips of the loins were equally represented in each of the cooking treatments.

Loins were cooked in a Southbend convection oven. Roasting and braising were performed in the same oven at 165° C to prevent oven variation. Roasted loins were cooked on a rack in stainless steel pans to an internal temperature of 75° C, while braised loins were cooked to an internal temperature of 85° C on a rack in stainless steel pans with 750 mL H_2O added per pan. These are the recommended endpoint temperatures for roasted and braised pork loin (Amer. Meat Sci. Assn., 1978). Foil was placed on the braising pans to prevent moisture loss. Raw loins were kept refrigerated while the other loins were being cooked. After cooking, loins were weighed and then refrigerated until all loins were cooked.

Loin sections were cut into small cubes and ground twice through a large-screen Hobart stainless-steel grinder with a plate containing 5 mm holes. After weights were obtained, the ground loins were placed on large stainless steel trays and frozen (−34° C) overnight. The loins were then freeze-dried for 24 hr after which they were ground twice in a Fitzmill (screen size 9 mm) to a fine powdery consistency. Weights were measured after grinding. Loins were analyzed for Zn by wet ashing using HNO_3 and H_2O_2 (Wedekind and Baker, 1990); Zn concentration was measured by atomic absorption spectrophotometry (Perkin-Elmer, Model 306; Perkin-Elmer Corp., Norwalk, CT). The freeze-dried finely ground pork loins contained 51, 60, and 63 mg Zn/kg and 4.10, 2.88, and 2.72% H_2O for the raw, roasted and braised loins, respectively.

Animals and diets

Two experiments were conducted using male chicks from the cross of New Hampshire males and Columbian females. Chicks were fed a corn-soybean meal starter diet (120 mg Zn/kg) from 1 to 7-day posthatching. On day-8 posthatching following an overnight fast, chicks were weighed and allotted to experimental groups so that each group had a similar average initial weight and weight distribution. Four (Exp. 1) or eight (Exp. 2) replicate groups of four chicks were fed the experimental diets from 8 to 22-days posthatching. Diets and deionized water were provided ad libitum. Chicks were kept on a 24 hr constant-light schedule in heated, thermostatically controlled stainless steel batteries with raised wire floors. Stainless steel waterers and feeders were also used to minimize Zn contamination.

The basal diets (Table 1) were formulated to contain adequate amounts of all nutrients except Zn. The Zn-unsupplemented corn-soybean meal basal diet contained 40.3 mg Zn/kg, just meeting the chicks' growth requirement for Zn of 40 mg Zn/kg (NRC, 1984). The Zn-deficient soy concentrate diet contained 14 mg Zn/kg. Dietary sources of Zn were added to diets at the expense of dextrose (soy concentrate diet) or cornstarch (corn-soybean meal diet). The Zn was provided as feed-grade $ZnSO_4 \cdot H_2O$ (36% Zn; Southeastern Minerals, Inc., Bainbridge, GA) or from pork loin.

Upon termination of experiments, chicks were killed via cervical

Table 1—Composition of the basal diets

Ingredient	Soy concentrate[a] %	Corn-soybean meal[b] %
Soy concentrate[c] (65% CP)	34.47	–
Corn (8.5% CP)	–	39.71
Soybean meal (48.0% CP)	–	40.89
Cornstarch	–	10.33
Dextrose	54.38	–
Corn oil	5.00	5.00
Zn-free mineral mix[d]	5.35	–
Dicalcium phosphate	–	2.20
Limestone	–	1.00
Iodized salt	–	0.40
DL-methionine	0.40	0.20
Choline chloride	0.20	0.10
Vitamin premix[e,f]	0.20	0.10
$FeSO_4 \cdot 7H_2O$	–	0.05
Lincomycin premix[g]	–	0.01
Selenium premix[h]	–	0.01
DL-α-tocopheryl acetate (20 mg/kg)	+	–
Ethoxyquin (125 mg/kg)	+	–

[a] Contained 22.4% CP, 14 mg Zn/kg, 1.1% Ca and 0.55% available P.
[b] Contained 23% CP, 40.3 mg Zn/kg, 1.1% Ca and 0.55% available P.
[c] Procon (A. E. Staley Inc., Decatur, IL).
[d] Mineral mixture provided per kilogram of diet: $CaCO_3$, 3 g; $Ca_3(PO_4)_2$, 28 g; K_2HPO_4, 9 g; NaCl, 8.8 g; $MgSO_4 \cdot 7H_2O$, 3.5 g; $MnSO_4 \cdot H_2O$, 0.65 g; $FeSO_4 \cdot 7H_2O$, 0.415 g; $CuSO_4 \cdot 5H_2O$, 20 mg; H_3BO_3, 9 mg; $Na_2MoO_4 \cdot 2H_2O$, 9 mg; KI, 40 mg; $CoSO_4 \cdot 7H_2O$, 1 mg; Na_2SeO_3, 0.215 mg.
[e] Vitamin premix for the soy isolate diet provided per kilogram of diet: thiamin · HCl, 20 mg; niacin, 50 mg; riboflavin, 10 mg; Ca-pantothenate, 30 mg; vitamin B_{12}, 0.04 mg; pyridoxine · HCl, 6 mg; biotin, 0.6 mg; folic acid, 4 mg; menadione, 2 mg; ascorbic acid, 250 mg; cholecalciferol (200,000 IU/g), 600 IU; retinyl acetate (650,000 IU/g), 5200 IU.
[f] Vitamin premix for the corn-soybean meal diet provided per kilogram of diet: retinyl acetate, 4400 IU; cholecalciferol, 1000 ICU; DL-α-tocopheryl acetate, 11 IU; vitamin B_{12}, 0.01 mg; riboflavin, 4.41 mg; d-pantothenic acid, 10 mg; niacin, 22 mg; menadione sodium bisulfite, 2.33 mg.
[g] Added to provide 4.4 mg lincomycin per kg diet.
[h] Added to provide 0.1 mg Se (from Na_2SeO_3) per kg diet.

dislocation and tibias were removed. Tibias were pooled by replicate, stripped of adhering tissue, and then dried at 105°C for 24 hr, weighed, and dry-ashed at 600°C overnight. The ashed bones were then wet ashed using HNO_3 and H_2O_2 (Wedekind et al., 1991). Zinc concentration was measured by atomic absorption spectrophotometry as described previously.

Zinc bioavailability experiments

Experiment 1 was conducted to determine the relative bioavailability of Zn in raw, roasted and braised pork loin compared to a $ZnSO_4$ standard. Supplemental levels of 0, 3.5 and 7.0 mg Zn/kg from $ZnSO_4$ were added to corn-soybean meal diets (Table 1) at the expense of cornstarch to construct a standard curve (total tibia Zn regressed on supplemental Zn intake). Tibia Zn responds linearly to Zn intake from $ZnSO_4$ at levels below 60 mg Zn/kg in corn-soybean meal diets (Wedekind et al., 1991). The standard curve was used to ascertain Zn bioavailability in pork loin based upon diets containing 4.8, 5.9, and 5.7 mg added Zn/kg diet from raw, roasted or braised pork loin, respectively.

Experiment 2 was conducted to determine the relative bioavailability of Zn in roasted pork loin and in $ZnSO_4$ in the presence of 0.4% L-cysteine. Supplemental Zn levels of 0, 5, 10 and 15 mg/kg from $ZnSO_4$ were added to the Zn-deficient soy-concentrate diet (Table 1) at the expense of dextrose. The standard curve was compared to diets containing 10 mg/kg Zn from roasted pork loin and from $ZnSO_4$ in the presence of 0.4% L-cysteine added to the diet.

Statistical analysis

All analysis of variance and regression analyses were conducted using the General Linear Model (GLM) procedure of SAS (1985). Total tibia Zn was regressed on supplemental Zn intake. Zinc bioavailability was determined relative to a $ZnSO_4$ standard using standard-curve methodology. The Y variable (tibia Zn content, μg) was substituted into the regression equation determined from the standard curve. Solving for X gave an estimate of the bioavailable Zn consumed. Dividing the bioavailable Zn consumed by the total supplemental Zn intake gave a relative bioavailability (%) estimate. Standard error values were determined, and treatment means were separated by the least significant difference (LSD) multiple pairwise-comparison procedure (Carmer and Walker, 1985).

Table 2—Efficacy of pork loin as a source of zinc for chicks fed a corn-soybean meal diet (Exp. 1)[a]

Supplement[b]	Suppl. Zn (mg/kg)	Weight gain (g)	Suppl. Zn intake (mg)	Tibia data: Dry wt (g)	Tibia data: Zn Conc. (μg/g)[c,d]	Tibia data: Total Zn (μg)[c,d,e]	RBV[e]
None	0	278	0	1.26	124.5[x]	157.5[x]	
$ZnSO_4$	3.5	287	1.39	1.27	150.9[y]	192.2[y]	
$ZnSO_4$	7.0	281	2.76	1.28	166.4[z]	213.3[z]	100
Pork loin (raw)	4.8	276	1.70	1.26	160.6[y,z]	202.8[y,z]	124
Pork loin (roasted)	5.9	292	2.20	1.29	168.8[z]	218.4[z]	131
Pork loin (braised)	5.7	290	2.12	1.30	165.4[z]	215.3[z]	129
Pooled SEM		4.1	0.05	0.03	4.5	7.9	16

[a] Data represent means of quadruplicate groups of four chicks during the period 8 to 22 days posthatching; average initial weight was 83 g.
[b] The pork loin products were freeze-dried after processing (i.e., cooking) and then finely ground. All sources of Zn were added to the corn-soy basal diet (40.3 mg Zn/kg) at the expense of cornstarch. Zinc concentrations in the freeze-dried pork loins were 51, 60 and 63 mg Zn/kg for the raw, roasted and braised products, respectively.
[c] Means within columns bearing different superscript letters (x, y, z) differ ($P < 0.05$).
[d] Linear response to $ZnSO_4$ addition ($P < 0.01$). Regression of tibia Zn (Y ln μg) on supplemental Zn intake from $ZnSO_4$ (X in mg) was $Y = 159.9 + 20.30X$ ($r = 0.84$).
[e] Standard-curve methodology was used to predict relative bioavailability (RBV) of roasted and braised pork loin. These estimates were not significantly different ($P > 0.05$) from 100%.

RESULTS

Experiment 1

Total tibia Zn and tibia Zn concentration both responded linearly ($P < 0.01$) to supplemental Zn intake from $ZnSO_4$ (Table 2). Linear regression of total tibia Zn (Y) on supplemental Zn intake (X) yielded the equation $Y = 159.9 + 20.30X$ ($r = 0.84$). Standard-curve methodology was used to predict the relative Zn bioavailabilities of the pork sources compared with the inorganic standard. This method indicated bioavailabilities were 124% (raw), 131% (roasted) and 129% (braised pork), with no significant difference from each other or from 100% ($P > 0.05$).

Experiment 2

Chicks gained more ($P < 0.05$) weight when fed diets containing 10 mg Zn/kg from pork loin or from $ZnSO_4$ plus L-cysteine than those fed the other diets (pooled comparison). Tibia Zn levels (both total and concentration) were greater ($P < 0.01$) in chicks fed 10 mg Zn/kg from pork loin or from $ZnSO_4$ plus L-cysteine than in chicks fed an equivalent level of Zn from $ZnSO_4$ alone. Total tibia Zn and tibia Zn concentration both responded linearly ($P<0.01$) to supplemental Zn intake from $ZnSO_4$ (Table 3). The linear regression of total tibia Zn (Y) on supplemental Zn intake (X) was $Y = 97.1 + 14.51X$ ($r = 0.87$). Standard curve methodology predicted relative bioavailabilities of the two experimental Zn sources compared with the $ZnSO_4$ standard (100%) of 173% for $ZnSO_4$ plus L-cysteine and 149% for roasted pork loin. Both values were greater ($P < 0.05$) than 100%.

DISCUSSION

FOOD SOURCES high in protein are the best sources of Zn (Prasad, 1983). Meat, fish and poultry are the major food sources of zinc, comprising an estimated 50% of the Zn in American diets (Moser-Veillon, 1990). Beef contains about 65 mg Zn/kg wet weight (National Live Stock and Meat Board, 1990) and has been shown to have a high Zn bioavailability. In a study by Shah and Belonje (1984) the bioavailability of

Table 3—Utilization of zinc by chicks fed a soy concentrate diet containing supplemental zinc from zinc sulfate, zinc sulfate plus cysteine, or pork loin (Exp. 2)[a]

Supplement[b]	Suppl. Zn (mg/kg)	Weight gain (g)[c]	Suppl. Zn intake (mg)	Tibia data: Dry wt (g)[c]	Tibia data: Zn Conc. (μg/g)[c]	Tibia data: Total Zn (μg)[c,d]	RBV[c,d]
None	0	236[x]	0	1.04[x,y]	87.1[v]	90.1[v]	
$ZnSO_4$	5	235[x]	1.83	1.03[x]	129.4[w]	132.7[w]	
$ZnSO_4$	10	244[x,y]	3.72	1.07[y,z]	142.0[x]	151.7[x]	
$ZnSO_4$	15	241[x]	5.54	1.01[x]	178.9[y]	174.8[y]	100[y]
$ZnSO_4$ + 0.4% L-Cys[e]	10	251[y,z]	3.72	1.04[x,y]	182.3[y]	189.6[z]	173[z]
Pork loin[f]	10	262[z]	3.48	1.03[x,y]	169.9[z]	176.1[y]	149[z]
Pooled SEM		2.6	0.04	0.01	2.5	3.2	13

[a] Data represent means of eight pens of four chicks during the period 8 to 22 days posthatching; average initial weight was 74 g.

[b] The basal diet contained 14 mg Zn/kg; additions were made at the expense of dextrose.

[c] Means within columns bearing different superscript letters (v-z) differ ($P < 0.05$).

[d] Linear regression of total tibia Zn (Y in μg) on supplemental Zn intake (X in mg) was $Y = 97.1 + 14.51X$ ($r = 0.87$). Standard-curve methodology was used to calculate relative bioavailability (RBV).

[e] Provided as L-cysteine · HCl · H_2O.

[f] Pork loin was roasted, freeze-dried and then finely ground prior to incorporation into the diet. The Zn concentration of the freeze-dried roasted pork loin was 60 mg/kg.

Zn in freeze-dried ground beef was 104% when fed to rats in an egg-white diet. The criterion measured was $\log_{10}$ total femur Zn (μg), and $ZnSO_4$ was the standard. Another study done with freeze-dried cooked beef indicated that Zn bioavailability in beef was equal to that in $ZnCO_3$ when fed to rats in an egg-white diet (Brown et al., 1985). Zinc absorption from a lactalbumin diet with added zinc sulfate showed equal absorption compared with Zn from a bologna-type meat emulsion (Greger et al., 1984). The distribution of Zn from the meat-based diet was greater throughout the body, with more accumulation in the liver. The authors indicated this was due to differential chelation of Zn in the body.

The bioavailability of Zn from raw and cooked pork loin in the first experiment (wherein a complex corn-soybean meal diet was fed) was not significantly greater than that of the $ZnSO_4$ standard. Zinc concentration in the cooked pork loin (26 mg Zn/kg average) was lower than that reported for other cuts of pork and for beef (National Livestock and Meat Board, 1990). The relative bioavailability estimates showed a tendency for Zn in pork loin to be more bioavailable than that which has been reported for beef. In experiment 2, roasted pork loin showed a significantly greater relative bioavailability (149%) than $ZnSO_4$ (100%). Zinc sulfate, whether $ZnSO_4 \cdot H_2O$ or $ZnSO_4 \cdot 7H_2O$, is considered an excellent Zn standard and, in fact, provides bioavailable Zn almost twice as efficiently as ZnO (Wedekind and Baker, 1990).

It has been suggested that chelates between animal protein and Zn may protect the Zn from inhibitory factors in the diet (Solomons and Jacob, 1981). Possibly pork may contain a Zn absorption-enhancing factor that is not present (or in lower concentration) in other sources of Zn. Amino acid ligands have been proposed as "enhancers" of Zn absorption.

Our study showed that adding L-cysteine together with $ZnSO_4$ in a soy concentrate diet increased Zn bioavailability. Other research has indicated that Zn from a Zn-methionine complex was more bioavailable than $ZnSO_4$ (Wedekind et al., 1991). With rats fed lactalbumin or soy-based diets, Zn absorption was significantly affected by dietary protein as well as the cysteine content of the protein (Snedeker and Greger, 1983; Greger and Mulvaney, 1985). That work did not show a significant effect of histidine on Zn absorption. Histidine may have a minor effect on Zn absorption, but results have been variable (Hortin, 1991). Layrisse et al. (1984) and Taylor et al. (1986) have suggested that enhanced nonheme iron absorption from meat was due to cysteine present in either free form or as cysteine-containing peptides. Sulfhydryl-containing compounds in pork such as cysteine or reduced glutathione may be postulated to participate in the "meat factor" responsible for enhanced bioavailability of Zn and iron in meat products (Layrisse et al., 1984; Greger and Mulvaney, 1985).

CONCLUSIONS

Zinc from pork loin provided more bioavailable Zn than the inorganic sulfate standard; adding L-cysteine to the diet greatly enhanced Zn absorption. Pork is a valuable source of bioavailable Zn; cysteine, and possibly other sulfhydryl compounds, are probably associated with the proposed "meat factor."

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Temperature, pH and Bacterial Populations of Meat as Influenced by Home Freezer Failure

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ABSTRACT

Consumers are concerned about the fate of frozen meat products in home freezers when electrical power is interrupted. We investigated this concern by using an upright home freezer. Fourteen trials were conducted with packages of either beef or pork with varying trial load weights (Range = 43–128 kg). Meat samples were frozen, power was disconnected, and packages within each lot were analyzed daily for 9 days for temperature, microbial count, and pH. After about 36 hr without electrical power, bacterial populations reached log 6 and the meat product's wholesomeness for consumption or possible refreeze became questionable.

Key Words: meat, home freezer, temperature, pH, warming, thawing

INTRODUCTION

FREEZING is a common method of food preservation for retailed packaged foods and has, in many cases replaced traditional canning and curing of foods for the home (Jul, 1984). Today many consumers purchase fresh retail cuts of meat, place them in a freezer and later thaw before cooking. The number of consumers using home freezers is increasing. This is reflected by the fact that the consumption of frozen foods has been steadily increasing in well developed countries over the past few decades e.g. in 1981 the USA had a 40 kg increase in per capita consumption of frozen foods compared to an average of the ten-year period prior to 1981 and this trend seems to be continuing (Jul, 1984).

The temperatures observed in home freezers in the USA as quoted by Olsson and Bengtsson were as follows; Below −18°C, 30% of the households; between −18°C and −12°C, 37%; between −12°C and −7°C, 22%; and above −7°C, 11% of the households (Olsson and Bengtsson, 1972). Consumers seldom experience a spoilage-related problem with frozen meat which has been properly packaged and stored. When they encounter an extended electrical power outage or freezer malfunction they become concerned about the safety of frozen meat products. University meat extension specialists receive periodic calls from consumers or home economists seeking advice on how long frozen meat products are safe to keep after electrical power has been interrupted. Current advice has been that as long as the meat remains frozen it is safe to keep but once the meat has thawed, its safety for consumption is questionable.

Microorganisms are primarily responsible for the deleterious effects to meat tissue if the meat product has been temperature abused in any manner. The freezing of muscle tissue extends the lag phase of microbial proliferation and thus extends the shelf-life of the meat product (Marriott et al., 1980). However, when electrical power is disconnected from a freezer, the temperature begins to rise over time and thus enhances bacterial proliferation. The bacterial genera present is more critical to spoilage than is total microbial load (Marriott et al., 1980). The elevated temperatures and increased thaw time create additional proliferation of the bacterial genera and meat spoilage becomes evident i.e. discoloration, putrid-odors, proteolysis, etc.

Numerous studies have documented the freezing and thawing rates for retail cuts of meat. No publications on this problem could be located. Therefore our objective was to investigate this unique problem area. The results should serve as a foundation for future consumer and scientific references.

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MATERIALS & METHODS

THIS INVESTIGATION was carried out in 14 consecutive trials, 11 using pork and 3 with beef. Each trial consisted of storing fresh retail cuts of meat samples with varying trial load weights (Range = 43-128 kg) in a −29°C upright White Westinghouse Model FU211C (0.6 cubic meter capacity) freezer. The meat cuts were packaged in 1.8–2.3 kg packages. The meat packages were wrapped in Copco Polyfreeze™ (polyethylene-coated) freezer paper. Once the meat samples were completely frozen for 1 wk, the electrical power was disconnected and packages within each lot per trial were analyzed periodically for 9 days for temperature, pH, and total viable plate count.

Temperature was monitored using thermocouples attached to an Omega Model 555 temperature recorder. The thermocouple was positioned to monitor the meat core temperature. The ambient room temperature was 23°C ± 3°C.

The pH measurements of the meat samples were determined by using an immersion probe attached to a Corning Model 7 pH meter (Ockerman, 1985). The pH meter was standardized using a two point pH calibration method with pH 7.0 buffer and pH 5.0 buffer. A 10g meat sample was placed into a sterile stomacher bag along with 100 mL of distilled water. The bag was stomached for 1 min in the Stomacher Lab Blender 400 and the subsequent pH measurement recorded.

The microbiological examinations of the meat samples were determined using the Standard Plate Count procedure (Speck, 1984). Three portions were taken from the meat sample for analysis, 1 slice from the center and 2 from about one-third the distance from each end. From each sliced portion a 10 gram sample was added to 90 mL of sterile 0.1% peptone water in a sterile stomacher bag. Each 10g sample contained a fixed proportion (≈ 50:50) of meat tissue from the surface and interior portion of the sample. The samples were macerated for 2 min using the Stomacher Lab Blender 400. Serial dilutions were prepared according to standard procedures (Speck, 1984). Bacterial counts were enumerated for total colony forming units. Difco standard plate count agar was used to determine total plate counts (25°C, 106 hr).

Statistical treatment

The plate count data were transformed into logarithms and statistical analysis was performed by using the Statistical Analysis System (SAS, 1986). The data (Fig. 4, 5, 6, and 7) were derived using the General Linear Models (GLM) procedure of SAS and included a covariant in the linear model. (Figures 4 and 5 included temperature as a covariant and Fig. 6 and 7 included bacterial count and trial load weights, respectively, as covariants.) When temperature was the dependent variable (Fig. 5 and 6) we included the independent variables, species (beef or pork), trial load weights, days storage time, and their interactions. In Fig. 4 and 7, bacterial count was the dependent variable and species, trial load weights, days storage time, temperature and their interactions were the independent variables of the model.

RESULTS & DISCUSSION

AT DAY 0 the electrical power was disconnected from the upright freezer. The freezer door remained closed until meat

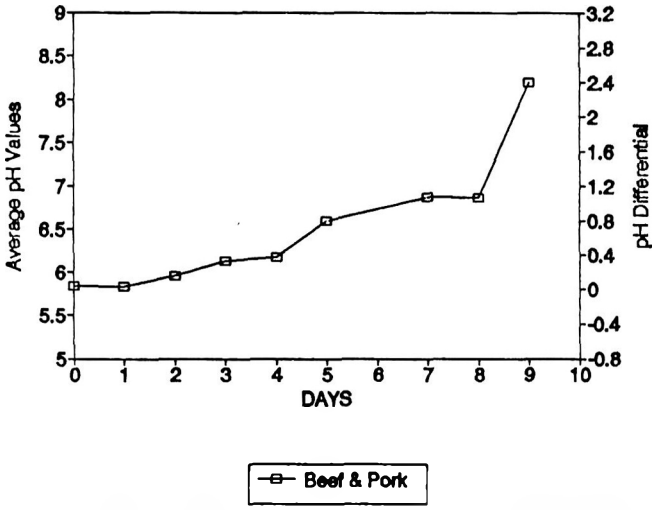

Fig. 1—Relationship between average pH and pH differential for all trials and time.

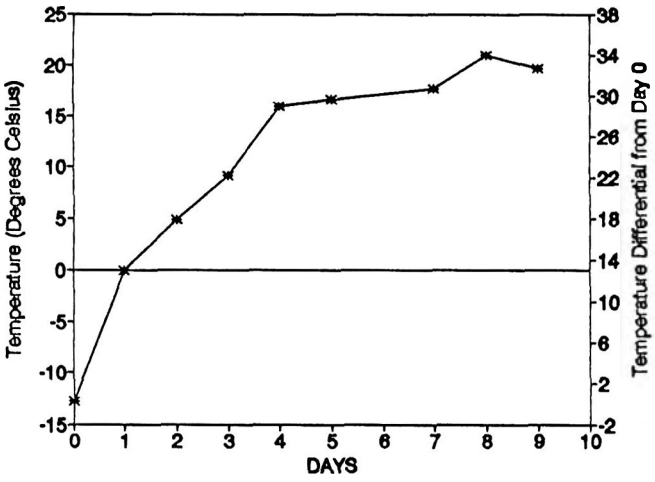

Fig. 2—Mean temperature for all samples and rate of temperature change.

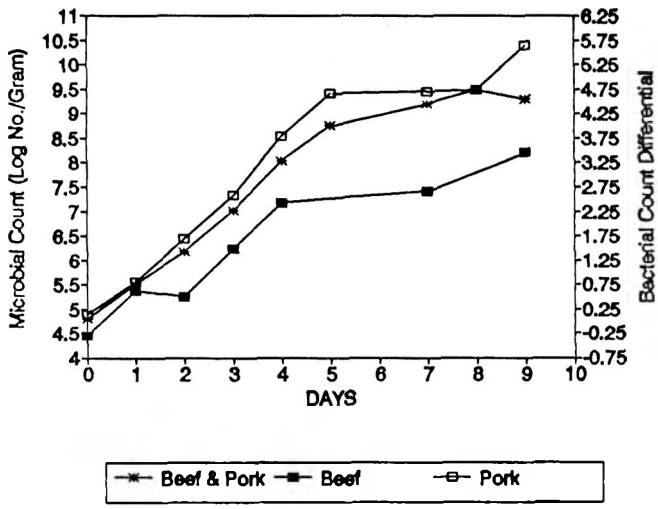

Fig. 3—Mean bacterial counts and rate of change in count during storage.

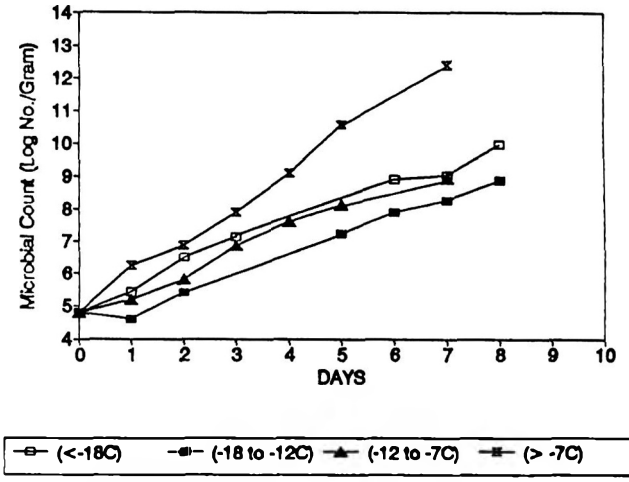

Fig. 4—Changes in mean bacterial count for the four initial temperature categories.

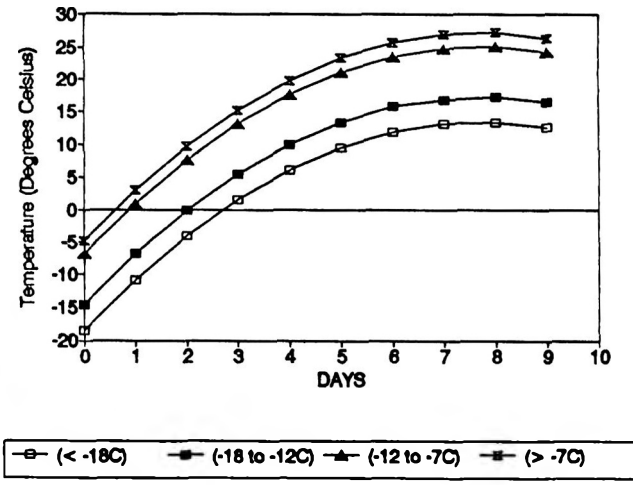

Fig. 5—Changes in mean temperatures of the four initial temperature categories with storage time.

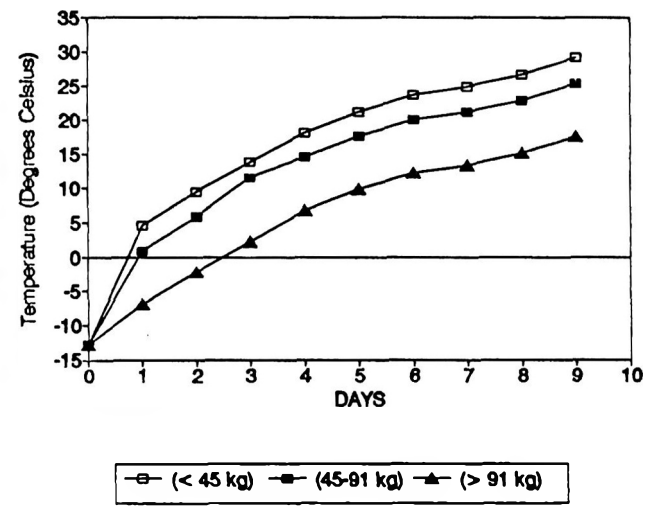

Fig. 6—Changes in mean temperatures for the three weight categories with time.

packages within each lot were removed and analyzed daily for temperature, bacterial count, and pH. The results revealed the pH of all samples (Fig. 1) increased from an average of 5.8 initially to 8.2 final. In Fig. 2 the mean temperature for all samples increased considerably during the term of the investigation. The rate of sample temperature increase averaged 5.8°C/day for the first 5 days, at which time the rate of temperature increase slowed and leveled off to the final sample temperature

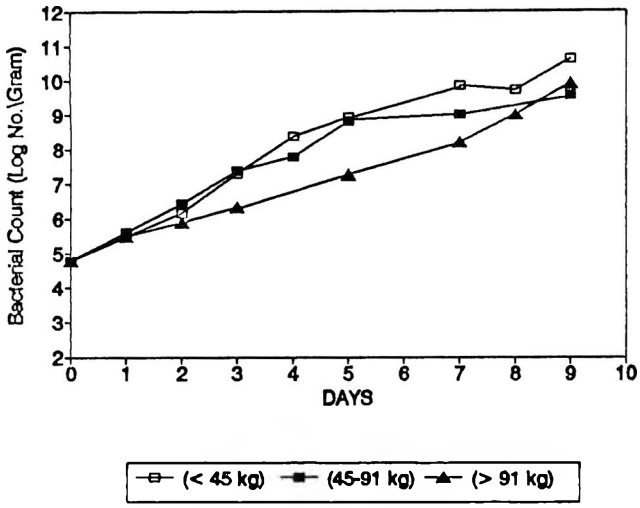

Fig. 7—Changes in mean bacterial counts for the three weight categories with storage time.

of 20°C at Day 9. The differential change in temperature from Day 0 to Day 9 was a 32.5°C increase.

As temperature increased, microbial growth increased at an accelerating pace. Figure 3 illustrates that the log bacterial counts for the combined beef and pork trials increased linearly up to Day 5 (0.79 log bacteria no./day), at which time the curve started to level off and the stationary phase was reached. At Day 9, the mean bacterial count was 4.5 log higher than the mean initial log count of 4.80 at Day 0. Figure 3 also shows that all pork samples had significantly higher bacterial counts than beef during the course of the study.

The trials were divided into the following temperature categories (below −18°C, −18° to −12°C, −12° to −7°C, and above −7°C) at Day 0 and all temperature categories were statistically adjusted to a mean bacterial count of log 4.91 at Day 0. In Fig. 4 the samples within each trial for each given temperature category were monitored for mean microbial count. The data reflect that the bacterial counts for the > −7°C samples had significantly higher bacterial counts over the other 3 temperature categories beginning at Day 3 of storage. Figure 5 shows the mean temperatures of the four initial temperature categories vs days of storage. All samples showed about the same rate of temperature increase during the study.

The trials were also divided into three weight categories (< 45 kg, 45–91 kg, > 91 kg) and were then adjusted to an average temperature at Day 0. Figure 6 illustrates the rate of temperature increase after Day 1 for the three weight categories were almost parallel. In Fig. 7, mean bacterial counts for the weight categories were monitored after adjustment to an average bacterial count at Day 0. Up to Day 5 there was no significant difference between categorical weight samples in microbial counts. At Days 5, 6 and 7 the lighter weight trials (i.e. <45 kg and 45–91 kg wt range trial groups) had significantly higher bacterial numbers than the heavier weight trial (> 91 kg). Both in Fig. 6 and 7 the mean temperatures and bacterial counts were adjusted to the same mean at Day 0.

At about 36 hr after loss of electrical power, bacterial counts reached log 6 from initial counts which averaged log 4.8. Log 6 is usually assumed to be a level at which meat products can be safely consumed so long as it is sufficiently cooked to destroy pathogenic organisms (Banwart, 1989). The USDA suggests a minimum end-point cooking temperature of 63°C. That temperature destroys some pathogenic microorganisms but will not affect bacterial spores, the enterotoxin of *Staphylococcus aureus* or the neurotoxin of *Clostridium botulinum* (Banwart, 1989).

CONCLUSION

IF A RETAIL MEAT product is frozen with a typical microbial load and the electrical power to the home freezer is interrupted for an extended time period (>36 hr), the frozen product would have a microbial count of about log 6 at around 36 hr after loss of power. Thus it would not be advisable to use the meat held for 36 hr after electrical power disconnect and the meat should probably be destroyed.

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Preblending Hot- and Cold-Boned Pork at Different Particle Sizes

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ABSTRACT

Hot- (HB) and cold-boned (CB) pork were ground through a 0.6, 1.3, or 1.9 cm plate, preblended (2% NaCl), and stored for 24 hr to evaluate resulting textural properties. Expressible moisture significantly decreased after storage, with HB preblends having less ($P<0.05$) expressible moisture than CB preblends. Peak extrusion force decreased ($P<0.01$) after storage for the largest particle size with no changes for other particle sizes. Kramer shear values decreased ($P<0.05$) as particle size increased, while a decrease in particle size effectuated an increase ($P<0.05$) in bind value. An increased quantity of salt during the preblending of HB meat may be advantageous to help achieve maximum protein extraction.

Key Words: Pork, preblending, hot-boning, cold-boning, protein-extraction, texture

INTRODUCTION

ACCELERATED PROCESSING has several advantages related to processing costs and product quality. Prior to the formation of actomyosin, larger quantities of myosin can be extracted which improves the binding and emulsifying properties (Hegarty et al., 1963; Hamm, 1975) of the raw material, resulting in a firmer texture for the finished meat product (Acton and Saffle, 1969; Hamm, 1975). Protein extraction is enhanced by grinding or chopping because of the disruption of the cell structure. Greater disruption of the cell structure with a concurrent increase in surface area has been shown to result in a greater release of the cellular components, and increased protein extraction (Schnell et al., 1970; Acton, 1972). Maximum extraction of protein is often desired to achieve optimal binding of fat and water when processing meat products.

The addition of salt to meat aids in extraction of the myofibrillar proteins, increasing binding and emulsifying properties (Hegarty et al., 1963). Mixing meat with salt and storing for a period of time (preblending) increases the functional properties of meat and has been used for many years. The improvement found in the functional properties of proteins extracted from preblended meat is attributed to enhanced salt penetration and increased protein-salt interactions (Ockerman and Crespo, 1982). In addition, when compared to preblended cold-boned meat, preblended hot-boned meat has shown enhanced water and fat retention properties (Hamm, 1975). The length of time postmortem, however, will decrease the advantage of hot-boned meat because of the advanced stage of rigor and the decline in pH.

Subjecting meat to preblending at smaller particle sizes should allow for increased salt penetration, thereby improving binding properties of the proteins. The objective of our study was to determine if advantages exist for preblending hot-boned meat at smaller grind sizes that would increase the binding capabilities of the raw material used for a coarse-ground sausage product.

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MATERIALS & METHODS

LACTATING SOWS ($n=16$) were separated prior to slaughter into 4 groups based on dietary treatment (Brendemuhl, 1985). Dietary regimens consisted of high protein, low energy; low protein, high energy; high protein, high energy; or low protein, low energy rations. One sow from each dietary regimen was placed into 1 of 4 groups to achieve equivalent composition among the groups. Each group represented 1 replication.

Hams were removed from the right side of each carcass and boned within 3 hr after exsanguination (HB). Left sides of the carcasses were chilled (4°C) for 48 hr prior to removal of the hams and subsequent boning (CB). After boning, the meat was ground (Model 4732, Hobart Mfg., Columbus, OH) through a 3-hole, kidney-shaped grinder plate and evenly divided among 3 particle size treatments (0.6, 1.3, 1.9 cm). Batches (6.3 kg) were ground through assigned plates, mixed (Model L100DA, Leland Detroit Mfg., Detroit, MI) (5 min) with 10% water (w/w), 2% salt (w/w) and 78 ppm sodium nitrite (50% of the total amount). Resulting preblends were stored for 24 hr (1°C) and subsequently mixed an additional 3 min to incorporate spices, 550 ppm sodium erythorbate and the remaining 78 ppm sodium nitrite. A final grind of 0.3 cm was used for all treatments prior to stuffing into 34 mm collagen casings. Sausages were heat processed in a smokehouse using 6 stages. In the first stage, the dry bulb was set at 54.4°C with no wet bulb setting for 30 min. The second stage was for 30 min with the dry bulb set at 60°C. During the third stage, which was for 30 min, the dry bulb was increased to 68.3°C with the wet bulb set at 54.4°C. A dry bulb setting of 73.9°C and a wet bulb setting of 62.8°C was held for an additional 30 min. The sausages were then steam cooked to an internal temperature of 66.7°C and subsequently showered with cold water for 15 min.

Raw preblend analyses

Expressible moisture was measured using the press method of Grau and Hamm (1953). A 0.3g sample was selected from each grind size, placed in the center of a dried Whatman #1 filter paper disk and pressed using 3600 kg pressure for 3 min. Measurement of the meat area and the juice area were accomplished with the use of a compensating polar planimeter. Expressible moisture was determined as the result of triplicate samples for each treatment. Samples for EM were obtained prior to preblending and after storage.

A 10-g sample was taken from each treatment prior to preblending, after storage and prior to stuffing. Each sample was blended with 100 mL 0.005M iodoacetate at high speed for 1 min to determine pH. The homogenate was decanted to a 150 mL beaker with a stir bar and measured with a pH meter (Orion, Cambridge, MA). The pH data reported was the mean of all measurements since there was no influence of sample point on the measured value.

A wire bar (0.25cm o.d. with edge to edge spacings of 0.5 cm) food extrusion cell (Conners Machinery, LTD., Sunco, Ontario, Canada) attached to an universal testing machine (Model 1123, Instron Corporation, Canton, MA) was used as described by Voisey and Larmond (1971) to measure viscoelastic properties. The sample cell was filled with 300 g of the meat mixture with efforts to minimize air pockets. Samples of the meat mixtures were extruded prior to preblending, after storage, and prior to stuffing. The mixtures were extruded at crosshead speed 50 mm/min with a 2:1 proportional chart speed. A 500 kg compression load cell with a load range setting of 0–50 kg was used to measure readings of peak force (kg).

Cooked product analysis

Moisture, protein, fat and ash contents were determined (AOAC, 1983). Moisture was determined by oven drying; protein was analyzed using Kjeldahl procedures; and fat was determined using Soxhlet procedures. Kramer shear force values were determined on two links

Table 1—Proximate analysis of coarse ground sausage as influenced by boning time and particle size

Boning time	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	pH[a]
Hot	59.7[c]	22.8[c]	15.3[c]	2.2[c]	6.32[c]
Cold	59.7[c]	22.7[c]	15.3[c]	2.1[c]	6.26[d]
s.e.[b]	0.2	0.1	0.1	0.1	0.01
Particle size (cm)					
0.6	59.8[c]	22.3[c]	15.4[c]	2.1[c]	6.29[c]
1.3	59.6[c]	22.8[c]	15.2[c]	2.1[c]	6.27[c]
1.9	59.7[c]	23.2[c]	15.2[c]	2.2[c]	6.27[c]
s.e.	0.5	0.4	0.2	0.1	0.01

[a] Mean of duplicate raw samples taken prior to preblending, after storage and prior to stuffing.
[b] s.e. = standard error.
[cd] Means within the same column and the same main effect with same superscript are not different (P > 0.05).

randomly selected from each treatment, casings removed, and cut to 4.5 cm. The links were placed side by side in the sample chamber such that the long axis was perpendicular to the orientation of the blades. Triplicate samples (6 links) were sheared for each treatment. A 250 kg load cell with a load range of 0–200 kg was used. Crosshead speed was 50 mm/min with a 2:1 proportional chart speed. Peak force (kg), and area under the curve (cm2) were obtained. Area under the curve was measured using a compensating polar planimeter.

Objective measurements for hardness, cohesiveness, elasticity, and chewiness were determined following the procedures of Friedman et al. (1963). Three sausage links per treatment were allowed to equilibrate to room temperature and sliced to a length of 3 cm. Each sample was oriented so that the long axis was parallel to the plane of compression. Each sample was compressed twice to 75% of its original thickness. A 250 kg load cell with a load range setting of 0–100 kg was used. Crosshead speed was 500 mm/min with a 2:1 proportional chart speed.

Statistical analysis

The study was designed as a randomized split-plot (Federer, 1955). Main effects (CB vs HB) and subplot (grind size) treatments were tested using least squares analysis of variance with multiple comparisons performed using Fisher's least significant difference test (SAS Institute Inc., 1988).

RESULTS & DISCUSSION

MOISTURE, fat, protein and ash contents of cooked sausage were not different (P > 0.05) for boning time or particle size (Table 1). Grouping the sows by nutritional regimen minimized the compositional differences between groups. Measurements obtained, therefore, were not appreciably affected by composition. Differences in pH between hot- and cold-boned pork were significant, but small (Table 1). When pork has substantially depleted stores of glycogen prior to exsanguination, usually brought about by long periods of stress, the pH of the muscle remains relatively high. Briskey et al. (1960) found that unusual nutrition was one cause for stress in animals. These data indicate that the HB muscle was in the advanced stages of glycolytic activity but had not achieved the ultimate pH exhibited by the CB meat.

A decrease in expressible moisture (EM) indicates an increase in the ability of the meat to retain moisture. Data in Table 2 indicated no change (P > 0.05) in EM due to boning time prior to storage. That hot-boned meat has a greater ability to retain moisture then cold-boned meat is well documented (Hamm, 1960; Acton and Saffle, 1969), but it is dependent on the pH of the muscle. Rigor development does not have a notable effect on the WHC of muscle due to the formation of crossbridges as the pH drops, decreasing the effect of rigor development (Hamm, 1986). As the data in Table 1 indicate, meat that was boned after 48 h was only slightly lower (P < 0.05) in pH than hot-boned meat. However, data in Table 2 indicate that the addition of salt and storage for 24 hr significantly

Table 2—Expressible moisture as influenced by boning time and sampling point[a]

Boning time	Before storage	After storage
Hot	62.9[b]	34.4[d]
Cold	60.2[b]	37.8[c]

[a] Standard error = 1.1.
[bcd] Means with different superscripts are different (P < 0.05).

Table 3—Effect of boning time, particle size, and sampling point on extrusion peak force values[a] (kg)

Boning time	Sampling time	Particle size (cm) 0.6	1.3	1.9
Hot	Before storage	17.4[e]	26.8[d]	55.1[b]
	After storage	15.7[e]	26.6[d]	45.4[c]
	Ground (0.3 cm)	13.7[e]	14.1[e]	13.3[e]
Cold	Before storage	17.4[e]	30.5[d]	50.2[c]
	After storage	16.8[e]	31.1[d]	34.5[d]
	Ground (0.3 cm)	14.2[e]	14.5[e]	15.0[e]

[a] Standard error = 2.0.
[bcde] Means within rows and columns with different superscripts are different (P < 0.05).

decreased expressible moisture in both hot- and cold-boned preblends, with hot-boned preblends retaining more (P < 0.05) moisture than cold-boned preblends. These data indicated that the ability to retain moisture was superior for the HB pork in the presence of salt. There was no significant interaction with particle size. Likely the 24 hr storage period was long enough for salt to migrate throughout even the largest meat particle. Acton (1972) found an increased ability for moisture retention with a decrease in particle size for salted meat not stored.

Extrusion peak force increased (P < 0.01) as particle size increased for both hot- and cold-boned pork (Table 3). This was expected since the spacings through which the meat was extruded did not change and the larger particles would require more force to be extruded through those spacings. Extrusion peak force was also affected by the structural integrity or pliability of the meat particles, with a reduction in force suggesting an increase in disruption of the structure. Data in Table 3 suggest no measurable change in pliability occurred during storage of salted meat ground to a particle size less than 1.9 cm since no difference (P > 0.05) in peak force values were indicated. It can be assumed, therefore, that structural integrity of the smaller particles was disrupted past the point of measurement by the extrusion test during mixing and that further disruption did not occur over the storage period. Extrusion peak force values decreased for the 1.9 cm particle size with a larger decrease (P < 0.05) observed for cold-boned meat when compared to hot-boned meat. The decrease in peak force values of samples after the storage period when compared to the samples prior to storage was assumed due to increased pliability of particles caused by salt penetration. As earlier suggested in Table 2, hot-boned meat possessed a greater ability to retain moisture, and, according to Acton and Saffle (1969) a greater amount of myosin was available in hot-boned meat. Salt and protein interactions in hot-boned meat may be maximized a shorter distance from the surface of the particle when compared to cold-boned meat due to less actomyosin formation. This is suggested by the data in Table 3 where the peak force value for CB was smaller (P < 0.05), indicating a higher degree of penetration. Actomyosin formation is the result of electrostatic linkages between actin and myosin (Hamm, 1986). Therefore, some of the binding between actin and myosin that occurred during the onset of rigor may have hindered the association between protein and salt. An increased amount of salt added to hot-boned meat during preblending may allow for greater penetration and improved protein extraction.

Kramer shear values were higher (P < 0.01) for HB sausage products (Table 4). Kramer shear values are an indication of tenderness which, for a ground meat product, is due to a com-

Table 4—Texture profiles of smoked and cooked sausage as affected by boning time and size of grind[a]

Variable	Boning time			Particle Size (cm)			
	Hot	Cold	s.e.[c]	0.6	1.3	1.9	s.e.
Kramer:							
shear, (kg/g)	1.01[d]	0.93[e]	0.01	1.00[d]	0.98[d]	0.94[e]	0.01
area, (cm2)	74.3[d]	72.6[d]	0.7	74.7[d]	73.9[de]	71.5[e]	0.9
Compression:							
Hardness, (kg)	22.1[d]	22.7[d]	0.4	22.6[d]	22.6[d]	22.0[d]	0.5
Elasticity	2.0[d]	2.0[d]	0.1	1.9[d]	2.0[d]	2.1[d]	0.1
Area, (cm2)[b]	9.8[e]	21.2[d]	0.3	21.1[d]	20.5[de]	19.9[e]	0.4
Cohesiveness	0.2[d]	0.2[d]	0.01	0.2[d]	0.2[d]	0.2[d]	0.01
Chewiness	9.1[d]	9.5[d]	0.3	8.9[d]	9.7[d]	9.3[d]	0.3

[a] Means of 4 batches/treatment heat processed using 2 replications.
[b] Area under the initial compression curve.
[c] s.e. = standard error.
[de] Means within main effect with similar superscripts are not different ($P > 0.05$).

bination of muscle structure and bind formation. The higher ($P < 0.01$) Kramer shear values shown by the HB pork, when compared to CB pork, may have been the result of less structural disruption as indicated. As the number of protein-salt interactions increase, the distance that salt can penetrate into the particle becomes limited by the initial salt concentration. Kramer shear values decreased ($P < 0.05$) as particle size increased (Table 4). In our case, Kramer shear values may be more an indication of bind formation since the particle size of the final product was the same for all treatments. Acton (1972) found an increase in bind formation with a decrease in particle size, primarily due to greater disruption of particles and increased protein extraction. Area under the Kramer shear curve also decreased ($P < 0.05$) with an increase in particle size.

Compression data indicated that only area under the first curve was influenced by boning time. Other measurements were not different. Area under the first curve appeared to be associated with bind formation. A higher value ($P < 0.05$) for hot-boned meat is indicative of greater bind formation due to extraction of myosin. Smaller values ($P < 0.05$) for compression area under the curve as particle size increased suggested a decrease in bind and substantiated our hypotheses concerning Kramer shear values. The compression values for area under the curve also indicated that the HB samples had less bind than did the CB samples, following similar trends as the Kramer shear data.

Our data suggested that a particle size less than 1.9 cm did not improve moisture retention for either hot- or cold-boned samples. Preblending smaller particle sizes increased the bind formation between particles in the finished product when compared to larger particle sizes. Pork preblended prior to chilling improved water-holding capacity when compared to CB pork, but maximum functionality may not be obtained using a 2% salt concentration. There may be an advantage in adding increased quantities of salt during preblending of hot-boned meat to take full advantage of protein extraction through increased salt/protein interactions.

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Rigor Condition, Tumbling and Salt Level Influence on Physical, Chemical and Quality Characteristics of Cured, Boneless Hams

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ABSTRACT

Influence of pre- or post-rigor conditions, short or long-time tumbling and 1.25 or 2.25% salt on the physical and chemical properties, quality and yield of hams were examined. Curing pre-rigor did not inhibit rigor, but long term tumbling slowed rigor development. Cell disruption and salt-soluble proteins extraction were enhanced more by processing pre-rigor than post-rigor. Pre-rigor intermittent tumbling for 18 hr produced a more uniform cured color and a more desirable slice appearance. Cohesiveness was improved by 18 hr intermittent tumbling. Bind strength at 6 hr intermittent tumbling of pre-rigor tissue was equivalent to that of 18 hr intermittent tumbling, suggesting that curing pre-rigor could reduce tumbling time.

Key Words: ham, rigor, tumbling, salt-level

INTRODUCTION

TUMBLING and massaging have been shown to improve the quality and yield characteristics of cured pork (Krause et al., 1978; Mills et al., 1980). Such mechanical agitation alters the muscle structure and aids extraction and solubilization of salt soluble proteins which bind meat pieces into one solid, uniform piece during cooking (Cassidy et al., 1978).

In an effort to reduce refrigeration costs, researchers have investigated curing of pre-rigor muscle tissue (Cross and Tennent, 1980; Ray et al., 1980; Henrickson, 1981). Results indicated improvements in cure migration, water-holding capacity and ultimate quality and yield characteristics when pre-rigor cured products are compared to similarly processed post-rigor muscle tissue (Mandigo and Henrickson, 1966; Arganosa and Henrickson, 1969; Mandigo and Kunert, 1973; Sefton, 1983; Perkins, 1984). Forrest et al., (1975) and Solomon and Schmidt (1980) demonstrated increased solubility of native muscle proteins due to the action of salt in pre-rigor processed meat, compared to post-rigor tissue. Hamm (1977) demonstrated that, in ground pre-rigor muscle tissue, salt inhibited post-mortem glycolysis.

Both pre-rigor and tumbled products are more pliable than post-rigor non-tumbled products, and flexibility normally enhances cure, penetration and uniformity. Whether pre-rigor curing reduced the amount of tumbling necessary to maximize quality while reducing refrigeration energy costs was studied by Henrickson (1981).

The objectives of our study were to determine the effects of rigor conditions in long- and short-time tumbled pork legs at two salt levels. Such effects were evaluated by changes in condition, salt soluble proteins (SSP), physical and meat quality factors.

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MATERIALS & METHODS

Forty market weight (91–118 kg) crossbred (Yorkshire, Duroc, Hampshire) hogs of similar genetic background were slaughtered at The Ohio State University Meat Laboratory. Following slaughter each carcass was either allowed to chill 12–15 hr (post-rigor) in a 2 ± 1°C cooler or the rear legs were removed pre-rigor for processing.

Prior to curing all rear legs were deboned, trimmed to 0.2 cm of fat cover and intermuscular fat removed. The pairs of legs were assigned to 1 of 5 processing treatment cells. These included either: (1) post-rigor curing and intermittent tumbling (10 min/hr) for 18 hr; (2) pre-rigor curing and holding for 18 hr; (3) pre-rigor curing and intermittent tumbling for 18 hr; (4) pre-rigor curing and intermittent tumbling for 6 hr and; (5) pre-rigor curing and continuous tumbling for 1 hr (Fig. 1). Tumbling times of 18 and 6 hr were selected to simulate industry practices of tumbling overnight or completing the curing process in an 8-hr shift. One-hour continuous tumbling was selected to maximize output with a minimum of equipment. Each tumbling treatment (nonvacuum) was subdivided into 2 salt levels of 1.25 and 2.25% salt.

The boneless legs (both pre- and post-rigor and both salt levels) were multiple stitch pumped (FOMACO, Model FGM 205, Copenhagen) to 25% above fresh weight with different percentages of sodium chloride in the brine (See Fig. 2) to obtain 1.25 and 2.25% of salt in the pumped products. Legs to be cured pre-rigor were multiple stitch pumped with a 4°C cure within 45 min. post-exsanguination and prior to tumbling in a 3°C cooler. Nontumbled legs were held 18 hr at 3°C in a closed container. At the end of the tumbling or holding period the legs were hand stuffed into a fibrous cellulose casing (Union Carbide F-9-EP, Chicago), tied and cooked in a smokehouse to internal temperature 66°C (See Fig. 2). After the internal temperature was reached hams were removed from the smokehouse and showered with cold tap water for 30 min before being moved to a 3°C holding cooler for 18 hr. The hams were then sampled.

Length of the biceps femoris muscle of the boneless leg was measured pre-cure (35 min postmortem). The differences between this length and the post-curing and tumbling length was expressed as change in muscle length. A muscle sample was obtained from the center of the semimembranosus muscle after curing and after tumbling. This tissue was fixed in formalin (10%), stained in haematoxylin and slides were prepared by The Ohio State University Department of Veterinary Pathobiology. Resulting slides were subjectively evaluated for cell membrane disruption, clarity of striation and disorganization of the nuclei according to the procedure of Cassidy et al. (1978). Muscle samples for all chemical analyses were removed from the semimembranosus muscle for chemical determinations prior to curing, post-curing and after tumbling or holding. Legs were also weighed each time and cooked hams were weighed again after 18 hr refrigeration (3°C).

Sample pH and water holding capacity were determined pre- and post-rigor in pre-cured, post-cured and post-tumbled and nontumbled legs according to the procedures outlined by Ockerman (1985). Salt soluble proteins were determined using the extraction procedure of Johnson and Henrickson (1970). The biuret method (Layne, 1957) was used to determine protein content.

Uniformity of cured color, cohesiveness/sliceability, sliced appearance, tenderness, and cured flavor of the cooked and cooled hams were evaluated by a trained six-member sensory panel using a 1 to 10 scale (10 extremely, 8 moderately, 6 slightly uniform, 4 slightly, and 1 extremely nonuniform in cured color; 10 extremely, 7 moderately, 4 slightly cohesive/sliceable and 1 not cohesive/sliceable; 10 extremely, 8 moderately, 6 slightly attractive, 4 slightly, and 1 extremely unattractive slice appearance; 10 extremely, 8 moderately, 6 slightly tender, 4 slightly, and 1 moderately tough; 10 extremely, 8

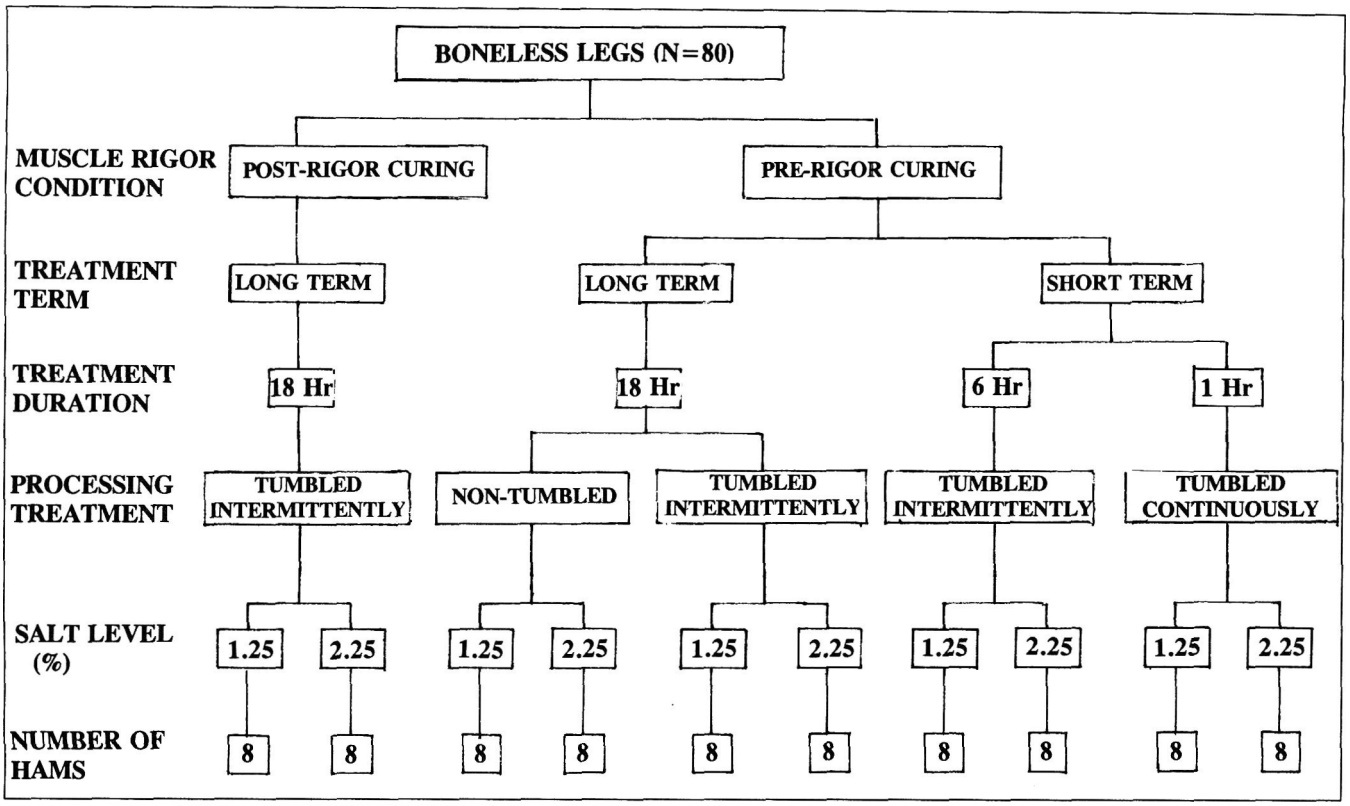

*Fig. 1—Experimental design. *Intermittent = 10 min/hr for total cycle.*

moderately, 6 slightly typical, 4 slightly, and 1 very abnormal cured flavor). Panel training consisted of 5 preliminary sessions in which extreme parameters were evaluated using the described techniques, and results were discussed. After training, 8 samples representing 2 treatments were evaluated per session each week for a 10-wk period in an air conditioned, fluorescent-lighted room in taste panel booths.

Binding strength and shear tenderness of the cooked hams were determined using the Warner-Bratzler attachment and a 2000g load cell for the Instron Universal Testing Machine. Shear values were measured in kg/cm^2 using 2.5 cm cores, crosshead speed 5 cm/min and chart speed 20 cm/min. Binding strength was determined using the method of Gillette et al. (1978) using gripper jaws to hold the slice in place. Bind values were reported in g/cm^2 using crosshead speed of 5 cm/min and chart speed 20 cm/min.

Each treatment was replicated with 8 hams from 8 different animals. Statistical analysis of results was completed using the Mixed Model Least Squares and Maximum Likelihood Program (Harvey, 1982). Raw boneless leg weights were regressed to the overall mean of 6.0 kg and percent pump was regressed to an overall mean of 24.56%. If the interaction was not significant the least squares mean was adjusted for the nonsignificant main effect and reported for the significant main effect. The pre-cure pH and the post-tumble pH were also treated in this manner, since published reports indicate that pH can have a major influence on other factors. The least square means were also statistically separated. If the interaction was significant then the data was listed in an interaction table showing the influence of all main effects.

Salt in pumped product (%)	1.25	2.25
Composition of cure (%) (125% pump)		
Salt (NaCl)	5.5	9.9
Water	90.2	85.8
Sodium tripolyphosphate	2.2	2.2
Sodium erythorbate	0.24	0.24
Sodium nitrite	0.069	0.069

Smokehouse schedule	Wet bulb (°C)	Dry bulb (°C)	Time
Drying	--	54	30 min
Smoking	--	54	1.5 hr
Cooking	63	76	4 hr
Cooking	64	82	to 66°C internal temperature

Fig. 2—Cure composition and smokehouse schedule for ham processing.

RESULTS & DISCUSSION

THE EFFECT of pre-rigor curing and tumbling time on the extent of rigor mortis in porcine muscle was shown (Tables 1 and 2) by changes in pH decline and in length of the biceps femoris muscle pre-cure to post-tumble. Pre-cure pH was lower ($P<0.05$) for post-rigor than for pre-rigor tissue (Table 1) as was expected. The 18 hr pre-rigor nontumbled tissue had a lower pH than the 18-hr pre-rigor tumbled tissue. A trend for muscle shortening in the biceps femoris, an indication of rigor development, (shown by negative values) was evident in all treatments. The greatest decrease, though nonsignificant, in muscle length (rigor) was noted in the pre-rigor cured but not tumbled treatment. Belousov et al. (1979) reported that tumbled treatments maintained the cured muscle in a "relaxed state characteristic of slaughter-warm meat." However, in our study neither curing nor tumbling prevented muscle shortening. Hamm (1977) reported that salt, when added to ground pre-rigor bovine muscle, inhibited rigor-mortis. The shortening of the biceps femoris muscle seemed to indicate that rigor mortis was not completely inhibited by curing. Note that the nontumbled tissue had the greatest shortening, followed by the 1-hr tumbled samples.

Additional evidence suggesting an influence of tumbling on rigor was noted in the interaction pH data (Table 2). All pre-rigor cured and tumbled treatments had higher post-tumble pH after tumbling than either the pre-rigor cured but nontumbled,

Table 1—Rigor condition-processing treatment effects on muscle, pH, water-holding capacity (WHC), salt soluble protein content (SSP), and histological evaluation[a]

Muscle rigor condition	Post-rigor curing		Pre-rigor curing							
Treatment duration			Long-term treatment				Short-term treatment			
Processing treatment	18 hr Tumbled[b]		18 hr Nontumbled[c]		18 hr Tumbled[b]		6 hr Tumbled[b]		1 hr tumbled[d]	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
pH Pre-cure	5.36[f]	0.07	6.23[g]	0.07	6.34[g]	0.08	6.27[g]	0.05	6.26[g]	0.07
pH Post-tumble	5.78[f]	0.04	5.66[g]	0.04	5.96[h]	0.05	6.02[h]	0.03	6.09[i]	0.04
WHC, pre-cure (% bound water)	70.7[f]	1.8	76.4[g]	1.8	80.6[h]	2.1	81.5[h]	1.3	76.6[g]	1.9
WHC, post-tumble (% bound water)	75.1[f]	1.5	83.7[g]	1.6	84.5[g]	1.9	89.3[h]	1.2	89.1[h]	1.6
SSP, pre-cure (mg SSP/gram sample)	62.5[f]	3.7	70.1[g]	4.0	69.3[g]	4.6	76.1[g]	2.8	76.1[g]	4.1
SSP, post-tumble (mg SSP/gram sample)	59.3[f]	2.2	65.3[g]	2.2	64.7[g]	2.6	70.9[h]	1.6	71.1[h]	2.3
Biceps femoris: (Change in muscle length, cm)	−0.91[f]	1.09	−2.24[f]	1.14	−0.99[f]	1.32	−0.61[f]	0.79	−1.19[f]	1.14
Clarity of striation[e]	1.4[f]	0.2	1.9[f,g]	0.2	2.2[g,h]	0.2	2.5[h]	0.1	2.4[h]	0.2

[a] Effects of salt level statistically absorbed, least-squares means (LSM) and standard errors (SE).
[b] Tumbled intermittently (10 min on, 50 min off).
[c] Held in covered container at 2 ± 1°C.
[d] Tumbled continuously.
[e] Score: 1.0 = clear; 3.0 = unclear.
[f,g,h,i] Least-squares means in the same row with different superscripts are significantly different ($P<0.05$).

Table 2—Salt level and rigor condition-processing treatment effects on muscle pH and histological evaluation[a]

Muscle rigor condition	Post-rigor curing				Pre-rigor curing															
Treatment duration					Long-term treatment								Short-term treatment							
Processing treatment	18 hr Tumbled[b]				18 hr Nontumbled[c]				18 hr Tumbled[b]				6 hr Tumbled[b]				1 hr Tumbled[d]			
	1.25%		2.25%		1.25%		2.25%		1.25%		2.25%		1.25%		2.25%		1.25%		2.25%	
Salt level	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
pH Pre-cure[f]	5.36	0.08	5.34	0.08	6.32	0.09	6.14	0.09	6.41	0.07	6.27	0.13	6.16	0.08	6.38	0.07	6.16	0.10	6.36	0.08
pH Post-cure[f]	5.53	0.08	5.69	0.08	6.37	0.08	6.25	0.08	6.56	0.08	6.40	0.12	6.26	0.07	6.44	0.06	6.25	0.09	6.40	0.07
pH Post-tumble[f]	5.79	0.05	5.78	0.05	5.61	0.05	5.72	0.05	6.03	0.04	5.90	0.08	5.91	0.05	6.13	0.04	6.06	0.06	6.13	0.05
Disorganization of nuclei[f,g]	1.19	0.08	1.13	0.09	1.63	0.09	1.41	0.09	1.46	0.07	2.08	0.13	1.25	0.08	1.59	0.07	1.36	0.09	1.23	0.08
Cell membrane disruption[f,g]	1.09	0.11	0.99	0.11	2.66	0.11	2.62	0.12	2.36	0.09	2.93	0.17	2.69	0.10	2.59	0.09	2.48	0.13	2.36	0.10

[a] Least-squares means (LSM) and standard errors (SE).
[b] Tumbled intermittently (10 min on, 50 min off) at 2 ± 1°C over the treatment period.
[c] Held in a covered container at 2 ± 1°C over the treatment period.
[d] Tumbled continuously at 2 ± 1°C.
[e] Salt level (%) in finished product.
[f] Rigor condition-processing treatment × salt level interaction significant ($P<0.05$).
[g] 1.0 = normal; 3.0 = abnormal.

or the post-rigor cured samples. The extent of pH decline was less in the pre-rigor cured, 18 hr tumbled hams than in those cured pre-rigor but not tumbled (average decline, both salt levels, −0.38 vs −0.57).

Cellular disruption possibly attributed to tumbling (Cassidy et al., 1978) might be involved in the reduction of pH decline. Tumbling enhances cure distribution and salt may retard enzyme action necessary for glycolysis and production of lactic acid. The pre-rigor meat that was injected and not tumbled decreased more in pH than the injected pre-rigor and tumbled meat. This suggested that there may not have been adequate distribution of ions in the nontumbled product to inhibit rigor mortis.

Three measures of cell disruption were evaluated: clarity of striation, disorganization of nuclei and cell membrane disruption (Tables 1 and 2). The pre-rigor cured treatment tissue samples, obtained after tumbling, were judged in general to be more abnormal than those from post-rigor cured and tumbled tissue based on those three measures. Obviously muscle cell structure was easier to disrupt by either the injection process of curing or by tumbling in pre-rigor compared to post-rigor muscle tissue.

Water-holding capacity (WHC) has been defined as the ability of meat to hold its own or added water during application of force or treatments such as grinding, processing or cooking. It is closely related to changes in muscle pH (Hamm, 1960). The lowest ($P<0.05$) WHC levels were found in the post-rigor cured treatments both for pre-cured and post-tumbled samples (Table 1). The highest post-tumbled WHC values were observed in pre-rigor cured, short term tumbled treatments. This was expected from pH data, and may reflect the fact that, in general, less cell membrane disruption (average 0.11 units) occurred in those short-term treatments compared to other pre-rigor treatments (Table 2).

Salt soluble protein (SSP) extraction is a positive result of tumbling and should be enhanced in pre-rigor muscle tissue. Trautman (1966) reported that a decrease in pH was followed by a linear decrease in protein solubility. Our results in general agreed with those findings (Table 1), but were not strictly linear. All pre-rigor treatments had greater ($P<0.05$) extractable SSP than the post-rigor curing treatment when sampled both pre-cure and after the tumbling treatment time. These results agreed with those of Acton and Saffle (1969) who reported 48% more SSP extracted in 3% NaCl for pre-rigor than post-rigor meat. Also Johnson and Henrickson (1970) found pre-rigor normal pH meat to contain 70% more salt soluble protein than post-rigor normal pH meat.

The post-tumbled samples for the short term tumbled pre-

Table 3—Rigor condition effects on ham cooked yield, slice sensory properties, binding strength and tenderness[a]

Muscle rigor condition	Post-Rigor Curing		Pre-Rigor Curing							
Treatment duration	Long-term treatment						Short-term treatment			
Processing treatment	18 hr Tumbled[b]		18 hr Nontumbled[c]		18 hr Tumbled[b]		6 hr Tumbled[b]		1 hr Tumbled[d]	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Cooked yield %[i]	88.5[h]	1.2	88.4[h]	1.3	88.5[h]	1.5	87.1[h]	0.9	85.1[i]	1.4
Cured color uniformity[e]	5.8[h,i]	0.3	5.6[h]	0.4	6.8[j]	0.4	6.2[i]	0.2	5.8[h,i]	0.4
Sliced appearance[e]	6.5[h]	0.3	5.0[i]	0.4	7.6[j]	0.4	6.5[h]	0.2	5.5[i]	0.4
Cured flavor[e]	6.9[h]	0.4	7.1[h]	0.4	6.9[h]	0.4	7.0[h]	0.3	7.1[h]	0.4
Cohesiveness/sliceability[e]	7.11[h]	0.24	3.93[l]	0.25	7.44[h]	0.29	6.05[k]	0.18	4.50[j]	0.25
Binding strength[g] g/cm²	825.88[h]	74.22	492.56[i]	76.96	789.77[h]	89.27	796.42[h]	54.38	570.22[i]	78.56
Panel tenderness[e]	6.4[h]	0.3	6.7[h]	0.3	6.3[h]	0.3	6.8[h]	0.2	6.6[h]	0.3
Instron tenderness[f] kg/cm²	4.11[h]	0.49	4.72[h]	0.50	3.52[h]	0.58	4.84[h]	0.36	4.54[h]	0.51

[a] Effects of salt level statistically absorbed, least-squares means (LSM) and standard errors (SE).
[b] Tumbled intermittently (10 min on, 50 min off) at 2 ± 1°C over the treatment period.
[c] Held in a closed container at 2 ± 1°C for the treatment period.
[d] Tumbled continuously over the treatment period.
[e] Score: 10 = Extremely uniform; 10 = Extremely attractive; 10 = Extremely typical; 10 = Extremely cohesiveness/sliceability; and 10 = extremely tender.
[f] Warner-Bratzler shear values; higher value = less tender.
[g] Force necessary for separation of binding junction.
[h,i] Least squares means in the same row with different superscripts are significantly different ($P<0.05$).
[i] Cooked yield = cooked weight/post-tumbled weight.

Table 4—Salt level and rigor condition effects on total cooked yield and slice cohesiveness[a]

Muscle rigor condition	Post-rigor curing				Pre-rigor Curing															
Treatment duration	Long-Term Treatment												Short-Term Treatment							
Processing treatment	18 hr Tumbled[b]				18 hr Nontumbled[c]				18 hr Tumbled[b]				6 hr Tumbled[b]				1 hr Tumbled[d]			
Salt level[e]	1.25%		2.25%		1.25%		2.25%		1.25%		2.25%		1.25%		2.25%		1.25%		2.25	
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE
Total yield[f,g]	106.8	1.6	108.2	1.7	98.8	1.7	104.1	1.7	107.6	0.4	102.3	2.6	107.8	1.5	104.8	1.4	101.7	0.1	106.6	0.0
Cohesiveness/ sliceability[f,h]	7.2	0.3	7.0	0.3	3.3	0.3	4.6	0.3	8.2	0.3	6.7	0.5	5.7	0.3	6.4	0.3	4.3	0.4	4.7	0.3

[a] Least-squares means (LSM) and standard errors (SE).
[b] Tumbled intermittently (10 min on, 50 min off) at 2 ± 1°C over the treatment period.
[c] Held in a covered container at 2 ± 1°C over the treatment period.
[d] Tumbled continuously at 2 ± 1°C.
[e] Salt level (%) in finished product.
[f] Rigor condition-processing treatment × salt level interaction significant ($P<0.05$).
[g] Weight after cooking divided by green weight × 100.
[h] Score: 1 = not cohesive; 10 = extremely cohesive.

rigor treatment had greater ($P<0.05$) SSP levels than the long term tumbled pre-rigor treatments. Note that during tumbling, the salt soluble protein in the muscle was solubilized and migrated to the muscle surface, with the degree of solubilization increasing as tumbling or massaging time increased (Siegel et al., 1978). In the short-term tumbled samples, less solubilization may have occurred. Hence, more SSP would remain for extraction.

Both WHC and SSP extraction are credited with affecting cooked yield in tumbled products. Cooked or smokehouse total yield (based on fresh weight with all legs absorbing the same percentage of brine) was lower ($P<0.05$) for the 1 hr tumbling treatment than for all other treatments (Table 3). Differences between the other short term treatment (pre-rigor cure, 6 hr tumbled) and the long-term treatments were found ($P<0.06$).

Quality of the finished, sliced product, when measured by sensory panel or objective Instron methods, was affected by the treatments (Table 3). Slices from hams processed pre-rigor and tumbled intermittently for 18 hr were found by the sensory panel to have more uniform cured color ($P<0.05$) than those from the same tumbling cured post-rigor (Table 3). The uniformity of cured color in the slices from the pre-rigor cured and 18 hr tumbled hams was also better than that from any other pre-rigor treatments. The effect of length of tumbling cycle on uniformity of cured color was consistent with reports of Ockerman and Organisciak (1978) and Krause et al. (1978).

The 18 hr tumbled pre-rigor treatment resulted in the most attractive slice appearance, followed by the 18 hr tumbled post-rigor and the 6 hr tumbled pre-rigor (which had identical scores). The sliced appearance of the 1 hr continuous tumble and the nontumbled treatments was the least desirable suggesting that intermittent tumbling was important to sliced appearance and that pre-rigor processing may reduce tumbling requirements. Cured flavor was not influenced by any treatment.

The primary function of tumbling, according to Viskase, Ltd. (1971), is to enable the binding of several large pieces of meat into one homogenous piece after cooking; i.e. to encourage slice cohesiveness and sliceability. Average cohesiveness/sliceability scores over both salt levels were as follows: nontumbled—3.93, 1 hr tumbled—4.50, 6 hr tumbled—6.05, 18 hr tumbled post-rigor—7.11 and 18 hr tumbled pre-rigor—7.44. These suggest an improvement in cohesiveness/sliceability values with increased tumbling time and a slight advantage for pre-rigor tumbling (Table 3 and an average score in Table 4). These cohesiveness/sliceability scores indicated that adequate protein solubilization did not occur in the nontumbled (value 3.9) and 1-hr continuous tumbled (value 4.5) products.

Binding strength was ranked similar to cohesiveness scores. Product tenderness whether measured by the panel or instrumentally was not affected by the treatments.

An interaction was found between rigor condition-processing treatments for total yield (based on finish weight) and cohesiveness/sliceability (Table 4). Values above 105 for total yield were found for post-rigor 18 hr tumbled at both salt levels, pre-rigor 18 hr tumbled at 1.25% salt level, pre-rigor 6 hr tumbled at 1.25% salt level, and pre-rigor 1 hr tumbled at 2.25% salt level. This suggested that tumbled and tumbling

time (up to 6 hr) in general increased yield. However, as significant interactions indicate influence of salt levels did not show a consistent effect.

Comparing cohesiveness/sliceability data the two 18 hr tumbling treatments were superior and the nontumbled and 1 hr continuous tumbled were the least desirable. The 6 hr tumbled product was intermediate as expected. There was no obvious pattern for influence of salt at these levels over the treatments evaluated. Cohesiveness was improved by both pre- and post-rigor 18 hr tumbling. Pre-rigor processing plus tumbling seemed to produce overall a more desirable product than the equivalent post-rigor tumbled product.

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Salmonella Detection in Meat and Fish by Membrane Hybridization with Chromogenic/Phosphatase/Biotin DNA Probe

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ABSTRACT

The hybridization specificity of a biotin labeled 1.8 kb HindIII DNA fragment was confirmed by colony hybridization with *Salmonella* and non-*Salmonella* isolates. Culture conditions were then tested for the enrichment of salmonellae in foods with large populations of competitive flora. Different conditions of preenrichment and selective enrichment could be used for detecting low populations of salmonellae in foods. Enrichment using lactose combined tetrathionate (CTET) broth and Salmonella-Shigella (SS) agar followed by spotting the suspected isolates on a nitrocellulose membrane [CETE→SS (S)] was better. As low as 1.6×10^0 salmonellae/g food could be detected.

INTRODUCTION

THE CONVENTIONAL METHOD for *Salmonella* detection includes multiple subcultural and biotype or serotype identification steps and may take 5–7 days. Several rapid methods have been developed and one of those methods is DNA hybridization (FDA, 1984; Swaminathan, 1985). This technique has been used in differential detection of various bacteria such as enterotoxigenic *E. coli* (Hill, 1981; Moseley, 1980), *Yersinia enterocolitica* (Hill et al., 1983), *Leishmania* spp. (Wirth and Pratt, 1982), *Staphylococcus aureus* (Notermans, 1988) and *Salmonella* spp. (Fitts et al., 1983; Fitts, 1985; Tsen et al., 1989).

Although DNA hybridization is a rapid method for Salmonella detection, the method is not reliable in detection of *Salmonella* in foods, such as ground beef, chicken, turkey etc., if only preenrichment steps are used prior to hybridization (Flowers et al., 1987). Detection of *Salmonella* in food samples, such as ground beef, pork, chicken, fish etc., especially when they are highly contaminated with microflora, has been difficult. For example, when foods, such as beef, fish, pork etc., purchased from conventional out-door markets, were detected with ^{35}S-labeled DNA probe, high numbers of salmonellae were required to give positive results when only preenrichment was used (Tsen et al., 1989). When ^{32}P-labeled DNA probe was used for *Salmonella* detection in foods, such as soy flour, macaroni etc., it could be detected after preenrichment (Fitts et al., 1983). However, detection of Salmonella in meats, such as ground beef, pork, chicken, turkey, fish etc., usually needed additional selective and post-enrichment steps. Thus 3 or 4 days were usually required to obtain DNA hybridization results (Emswiler-Rose et al., 1987; Flowers et al., 1987).

Although detection of *Salmonella* in foods with ^{35}S or ^{32}P-labeled DNA probes have been reported (Tsen et al., 1989; Flowers et al., 1987; Emswiler-Rose et al., 1987), its detection by membrane hybridization with nonradioactive DNA probes has not been reported. Recently a nonradioactive Gene-Trak system has been developed for detection of *Salmonella*, but DNA hybridization of this system was in solution rather than on nitrocellulose (NC) membrane (Chan et al., 1990; Curiale et al., 1990). Our objective was to investigate the possibility for detection of *Salmonella* in foods with large populations of competitive flora by membrane hybridization with non-radioactive DNA probe using several enrichment steps followed by hybridization with the biotin labeled probes. Procedures included preenrichment of salmonellae in foods with combined lactose tetrathionate (CTET) broth followed by streaking the culture on *Salmonella-Shigella* (SS) agar. The suspected bacteria were then spotted on NC membrane. Condition as described above, i.e., the CTET->SS (S) condition, could be used prior to membrane hybridization.

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MATERIALS & METHODS

Bacterial strains

The *Salmonella* strain used for DNA probe preparation was *S. typhimurium* (ATCC 14028). Various *Salmonella* serovars and some non-*Salmonella* isolates, including *Escherichia coli, Citrobacter, Shigella, Klebsiella* etc. were obtained from the United States Department of Agriculture (USDA), American Type Culture Collection (ATCC), the Food Industry Research and Development Institute (FIRDI, Sinchu, Taiwan), the Bureau of Food & Drug Administration (BFDA, Taipei, Taiwan), and National Ping-Tung College of Agriculture (Ping-Tung, Taiwan). Normally, a loopful of tested bacterial strains were inoculated into 5 mL of Luria broth (L broth: yeast extract, 5g; tryptone, 10g; NaCl, 5g and H_2O, 1000mL). After incubation at 37°C for 18 hr, a portion of the culture was diluted and plated on L agar for bacteria counts or for further transfer.

DNA-DNA hybridization with biotin labeled probe

Salmonella or non-*Salmonella* on the L agar plate as described above was transferred with sterile toothpicks onto a nitrocellulose (NC) membrane (0.45 μ Amersham Hybond-C Extra) laid on a fresh L agar plate. The plate was then incubated at 37°C until the colonies grew to about 2 mm diam. Conditions for treatment of the bacteria on the NC membrane and DNA hybridization were described in a previous report (Tsen et al., 1989) except conditions for proteinase K (20 unit per mg; Boehringer Mannheim Biochemia, W. Germany) treatment and washing conditions after hybridization were modified. Concentrations of proteinase K could be from 600 μg to 2 mg/mL. depending on time of treatment which could be varied from 2 hr to overnight. After hybridization with the biotin labeled 1.8 Kb DNA probe, the NC membranes were washed twice with 2×SSC (1×SSC is 0.15M NaCl plus 0.015M sodium citrate), 0.1% sodium dodecyl sulfate (SDS) (25°C, 5 min ea wash), twice with 0.2×SSC, 0.1% SDS (25°C, 5 min ea wash), twice with 0.16×SSC, 0.1% SDS (50°C, 20 min ea wash), then once with 2×SSC, 0.1% SDS for 2 min followed by moistening with buffer I (0.1M Tris-HCl, pH 7.5, 0.15M NaCl). The NC membrane was then transferred into buffer I containing 3% BSA and incubated at 65°C, for 1 hr. After removing the buffer solution, the NC membrane was incubated at room temperature with streptavidin-alkaline phosphatase solution for 10 min and then washed twice with buffer I (15 min ea wash), twice with buffer II (0.1M Tris-HCl, pH 9.5, 0.1M MgCl, 0.1M NaCl, 10 min ea wash). The membrane was then incubated at room temperature with nitroblue tetrazolium (NBT) and 5-Bromo-4-chloro-3-indolyl-phosphate (BCIP) solution prepared in buffer II for color development using the BRL Blue Gene System.

Hybridization sensitivity

Normally 5 mL of Luria broth (LB) was inoculated with 1 loop of salmonellae. After incubation at 37°C for 12 hr and serial dilutions with sterile water, salmonellae were collected on a NC membrane by filtration with Bio-dot apparatus (Bio-Rad). Filtration or retention of

Table 1—Hybridization of the biotin-labeled 1.8 kb DNA probe with various Salmonella *isolates*

Serotypes	No. of isolates	No. of positive results	Serotypes	No. of isolates	No. of positive results	Serotypes	No. of isolates	No. of positive results	Serotypes	No. of isolates	No. of positive results
Group O2(A)			*S. nigeria*	1	1	*S. anatum*	6	6	**Group O6,14(H)**		
S. paratyphi A	1	1	*S. ohio*	5	5	*S. give*	1	1	*S. boecker*	1	1
Group O4(B)			*S. oranienburg*	6	6	*S. lexington*	4	4	*S. bousso*	1	1
S. abortusequi	1	1	*S. richmond*	2	2	*S. london*	2	2	*S. florida*	2	2
S. agona	2	2	*S. tennessee*	7	7	*S. meleagridis*	1	1	**Group O16(I)**		
S. azteca	1	1	*S. thompson*	5	5	*S. munster*	3	3	*S. hvittingfoss*	2	2
S. bredeney	2	2	*S. virchow*	1	1	*S. nchanga*	1	1	**Group O18(K)**		
S. bardo	1	1	**Group O6,8(C2)**			*S. sinstorf*	1	1	*S. cerro*	12	12
S. cairo	1	1	*S. aba*	1	1	*S. vejle*	1	1	**Group O21(L)**		
S. california	1	1	*S. bovismorbificans*	1	1	*S. uganda*	1	1	*S. minnesota*	4	4
S. chester	1	1	*S. blockley*	1	1	*S. weltevreden*	1	1	*S. ruiru*	1	1
S. derby	3	3	*S. chailey*	1	1	**Group O3,15(E2)**			**Group O28(M)**		
S. eppendrof	1	1	*S. hadar*	16	16	*S. binza*	1	1	*S. pomona*	1	1
S. essen	1	1	*S. kuru*	1	1	*S. drypool*	5	5	**Group O30(N)**		
S. heidelberg	6	6	*S. litchfield*	3	3	*S. goerlitz*	1	1	*S. bietri*	1	1
S. indiana	3	3	*S. manhattan*	5	5	*S. halmstad*	1	1	**Group O35(O)**		
S. java	6	6	*S. muenchen*	1	1	*S. kinshasa*	1	1	*S. adelaide*	3	3
S. lagos	1	1	*S. newport*	3	3	*S. lanka*	1	1	*S. alachua*	3	3
S. limete	1	1	*S. tananarive*	1	1	*S. newbrunswick*	1	1	**Group O40(R)**		
S. paratyphi B	1	1	**Group O8(C3)**			*S. newington*	3	3	*S. allanadle*	1	1
S. preston	1	1	*S. albany*	3	3	*S. portsmouth*	1	1	*S. johannesburg*	10	10
S. reading	2	2	*S. bardo*	1	1	**Group O3,15,34(E3)**			**Group O42(T)**		
S. saintpaul	1	1	*S. emek*	1	1	*S. arkansas*	3	3	*S. gera*	2	2
S. sandiego	1	1	*S. haardt*	3	3	*S. illinois*	1	1	**Group O45(W)**		
S. sarajane	1	1	*S. molade*	1	1	*S. thomasville*	5	5	*S. dugbe*	1	1
S. schwarzengrund	4	4	*S. istanbul*	2	2	**Group O1,3,19(E4)**			**Group O48(Y)**		
S. stanley	1	1	*S. kentucky*	6	6	*S. chittagong*	1	1	*S. djakarta*	1	1
S. trachau	1	1	**Group O9,12(D1)**			*S. ngor*	1	1	**Group O67**		
S. typhimurium	11	11	*S. berta*	5	5	*S. senftenberg*	12	12	*S. crossness*	1	1
Group O6,7(C1)			*S. dublin*	2	2	**Group O11(F)**			**Group O50(Z)**		
S. bareilly	5	5	*S. enteritidis*	3	3	*S. aberdeen*	1	1	*S. wasssenaar*	1	1
S. bonn	1	1	*S. gallinarum*	1	1	*S. rubislaw*	2	2			
S. braenderup	6	6	*S. javiana*	1	1	**Group O13,22(G1)**					
S. brandenburg	2	2	*S. miami*	2	2	*S. poona*	1	1			
S. colorado	1	1	*S. moscow*	1	1	**Group O13,23(G2)**					
S. choleraesuis	1	1	*S. panama*	2	2	*S. cubana*	3	3			
S. coleypark	1	1	*S. seremban*	1	1	*S. havana*	7	7			
S. colindale	1	1	*S. typhi*	2	2	*S. worthington*	3	3			
S. hartford	1	1	*S. victoria*	1	1						
S. infantis	7	7	**Group O3,10(E1)**								
S. lille	2	2	*S. amager*	2	2						
S. livingstone	2	2									
S. mbandaka	3	3									
S. montevideo	8	8									
									Total	324	324

*Table 2—Hybridization of non-*Salmonella *isolates with the biotin-labeled 1.8 kb DNA probe*[a]

	No. of isolates tested
Enterobacteriaceae	
Citrobacter freundii	3
Enterobacter aerogenes	1
Erwinia carotovara	1
Escherichia coli	26
Hafnia alvei	1
Klebsiella pneumoniae	2
Kluyvera ascorbata	1
Morganella morgnaii	1
Proteus vulgaris	2
Shigella flexneri	1
Shigella sonnei	1
Yersinia enterocolitica	1
Other bacteria	
Acinetobacter calcoaceticus	1
Alcaligenes faecalis	1
Bacillus cereus	1
Bacillus stearothermophilus	1
Bacillus subtilis	1
Brevibacterium linens	1
Micrococcus roseus	1
Staphylococcus aureus	3
Total	51

[a] No result was positive.

bacteria by filters was evaluated by viable bacteria counts before and after filtration. Bacteria collected on the NC filters were then subjected to DNA-DNA hybridization as described above.

Standard plate counts

Viable bacteria counts were estimated with plate count agar (PCA). For bacteria counts in food samples, 1g of the minced food sample was mixed with a Vortex with 9 mL sterile water. After serial dilution with sterile water, bacteria numbers were estimated with PCA as well.

Bacteria culture for detection of salmonellae in foods

One gram minced food sample was mixed with 9 mL of L broth at room temperature. To this mixture, 100 μL sterile water with or without salmonellae ($10^0 \sim 10^8$) was added and the mixture was incubated at 37°C with shaking for 12–18 hr. This was labeled culture L. One mL of culture L was diluted fourfold with sterile water and bacteria in 0.2 mL diluted culture, was collected on NC membrane (0.45μ, Amersham Hybond-C Extra) by filtration using a Bio-dot apparatu (BioRad). These were termed as the LB (F) conditions.

One mL culture L from above was transferred to 9 mL of tet thionate (TT) broth (DIFCO, 1984) at room temperature. After i cubation at 43°C for 12 hr, 1 mL of culture was mixed with 9 m Gram negative (GN) broth and again incubated at 37°C for 12 b Bacteria in 0.2 mL of the twofold diluted culture was collected c NC membrane as described. This bacteria culture condition was termed LB→TT→GN (F). Also, one loop of bacteria culture from the TT broth was streaked on the *Salmonella-Shigella* Agar (SSA) plate and incubated at 37°C for 18–24 hr for further identification.

One gram of minced food was mixed with 9 mL of prewarmed (37°C) lactose combined tetrathionate broth (lactose broth, 13g; sodium thiosulfate, 30g; calcium carbonate, 10g; bile salts, 1g; H_2O, 1000 mL and iodine solution 2 mL/100 mL broth, added after boiling and cooling to 60°C (Sveum and Kraft, 1981). To this mixture, 100 μL sterile water with or without salmonellae ($10^0 \sim 10^8$) was added

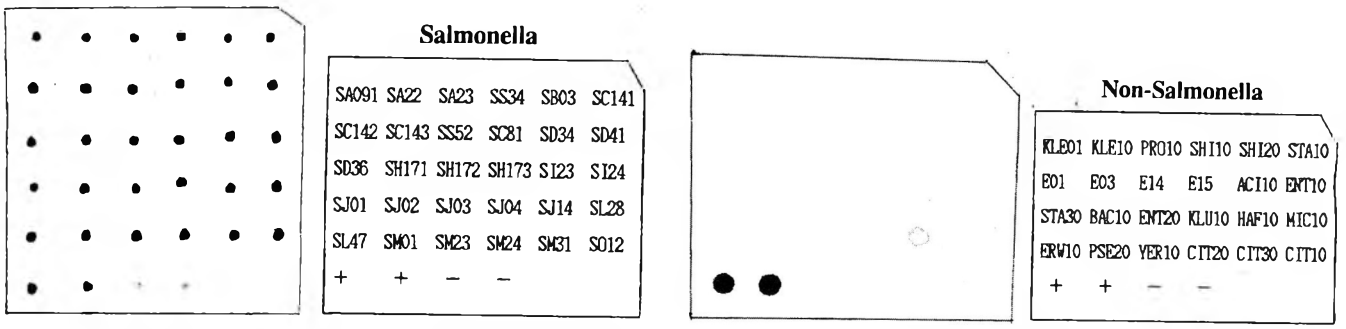

Fig. 1.—Colony hybridization patterns for some Salmonella *and non-*Salmonella *isolates with the biotin labeled 1.8 kb DNA probe. Experimental conditions were as described in Material and Methods. Bacteria for positive control: "+",* S. typhimurium *(ATCC 14028) and for negative control: "−"* E. coli. *Symbols for the colonies are as described in the following.*

Salmonella*: SA091,* S. adelaide*; SA22, SA23,* S. anatum*; SB03,* S. bareilly*; SB07,* S. berta*; SB51,* S. braenderup*; SC14, SC141, SC142, SC143,* S. cerro*; SC81,* S. cubana*; SD34, SD36,* S. drypool*; SD41,* S. dublin*; SH171, SH172, SH173,* S. havana*; SI23, SI24,* S. infantis*; SJ01, SJ02, SJ03, SJ04, SJ06,* S. java*; SJ14,* S. johannesburg*; SL28,* S. lille*; SL47,* S. livingstone*; SM01,* S. meleagridis*; SM23, SM24,* S. montevideo*; SM31,* S. muenster*; SO12,* S. ohio*; SS52,* S. schwarzengrund.

*Non-*Salmonella*: KLE01,* Klebsiella pneumoniae*; KLE10,* K. pneumoniae*; PRO10,* Proteus vulgaris*; SHI20,* Shigella sonnei*; STA10, STA30,* Staphylococcus aureus*; ACI10,* Acinetobacter calcoaceticus*; ENT10,* Enterobacter aerogenes*; ENT20,* Entrobacter cloacae*; BAC10,* Bacillus cereus*; KLU10,* Kluyvera ascorbata; HAF10, Hafnia alvie*; MIC10,* Micrococcus roseus*; ERW10,* Erwinia cartovora*; PSE20,* Pseudomonas cepacia*; CIT20, CIT30, CIT10,* Citrobacter*; YER10,* Yersinia enterocolitica*; EO1, EO3, E14, E15,* E. coli.

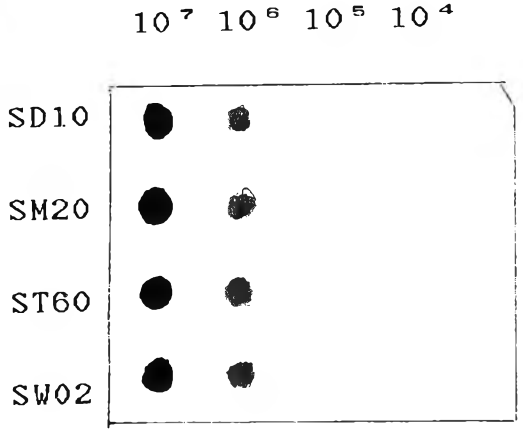

Fig. 2.—Hybridization sensitivity of the biotin labeled 1.8 kb DNA probe to some Salmonella *isolates. Experimental conditions were described in Materials & Methods. The bacteria numbers were shown as 10^4 ~ 10^7 per dot. Symbols SD10,* S. derby*; SM20,* S. muenchen; ST60, S. typhimurium*; SW02,* S. wassenaar respectively.

and the mixture then incubated at 43°C with shaking for 12 hr. 1 mL of this culture (CTET) was transferred to 9 mL GN broth and incubated at 43°C for 8 hr. After incubation, bacteria in 0.2 mL of the twofold diluted culture was collected on NC membrane by filtration. This was described as CTET (43°C) → GN (F) condition. Also, one loop of CTET culture (43°C) was streaked on the SS plate and incubated at 37°C for 18 hr. The suspected colonies of *Salmonella* on the SS plate were stuck with a sterile toothpick and spotted on the NC membrane laid on fresh L agar plates. The plates were then incubated at 37°C for 4 hr until the colonies on NC membrane grew to about 2 mm diam. This condition for bacteria culture was labeled CTET→SS(S) condition. Conditions for bacteria lysis and hybridization were as described above.

DNA probe

The 1.8 kb *Hind* III DNA fragment as the hybridization probe was isolated from a clone labeled H1.8. This clone was derived from a clone, Y3-1, which was obtained by transformation of *E. coli* with ligates of the BamH I digested total DNAs (2.5 Kb–8.4 Kb) from *S. typhimurium* and plasmid vector YEp 13. The transformants resistant to ampicillin but sensitive to tetracycline were than digested with *Bam*H I, Southern blotted and hybridized to the nick translated DNAs from *E. coli* or *S. typhimurium*. Although the 4.1 Kb insert in the Y3-1 clone could hybridize both *Salmonella* and *E. coli* DNA, a 1.8 Kb *Hind* III fragment derived from this 4.1 Kb fragment was found to hybridize with the *Salmonella* DNA only. Ligation of this 1.8 Kb fragment with *Hind* III digested YEp13 and transfered to E. coli would generate the H1.8 clone (Tsen et al., 1989). For probe use, the 1.8 Kb DNA *Hind* III fragment could be cut out of the plasmid from the H1.8 clone and nick translated with biotin-11-dUTP according to the method of Rigby et al., (1977).

RESULTS

Hybridization specificity and sensitivity for biotin labeled 1.8 kb DNA probe

In a previous report (Tsen et al., 1989) we have shown hybridization specificity of a 1.8 Kb DNA probe by membrane hybridization of the ^{35}S labeled probe with 50 domestic Salmonella isolates and some non-*Salmonella* isolates (Tsen et al., 1989). To use the nonradioactive DNA probe for detection of *Salmonella* in foods, hybridization specificity of the biotin labeled DNA probe was confirmed by colony hybridization with 324 *Salmonella* isolates of various serotypes including common and uncommon strains found in foods (Table 1). All the 324 *Salmonella* isolates could hybridize with this biotin labeled probe. In addition, under conditions described, DNAs of the 50 non-*Salmonella* isolates including Enterobacteriaceae closely related to *Salmonella* such as *E. coli, klebsiella, Citrobacter* and *Shigella* etc. did not show any hybridization signal with the nonradioactive DNA probe (Table 2). Part of the hybridization patterns are shown in Fig. 1 and the results were all clear and unambiguous. Therefore, hybridization specificity of this biotin labeled probe was assured.

Hybridization sensitivity studies for several common and uncommon *Salmonella* isolates including *S. derby, S. muenchen, S. typhimurium* and *S. wassenaar* showed that about 10^6 ~ 10^7 salmonellae per colony were required for positive detection of Salmonella and the hybridization levels for these *Salmonella* isolates were nearly the same (Fig. 2).

Enrichment and detection of *Salmonella* in foods highly contaminated with natural microflora

Effects of bacterial culture conditions on detection of *Salmonella* in foods using the biotin labeled DNA probe were investigated with beef and fish (Tilapia). Both samples were highly contaminated with endogenous microflora. For example, the viable bacterial counts were estimated at 3×10^6 or

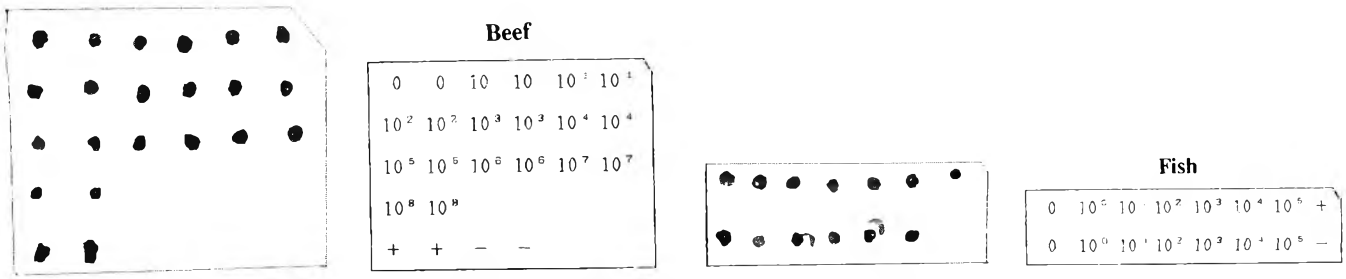

Fig. 3.—DNA-DNA hybridization patterns for detection of Salmonellae in Beef (I) and Fish (I) (Tilapia) samples with CTET→SS (S) culture condition. Experimental conditions were as described in Materials & Methods. Approximately 10^0 ~ 10^8 salmonellae were inoculated into 1g of the minced food sample before enrichment. Bacteria for positive control (+) S. typhimurium*; for negative control (−),* E. coli.

Table 3—Detection of Salmonella *in control and inoculated foods with biotin labeled DNA probe. The CTET→GN (F) culture condition*[a]

Food	SPC[b] (CFU/mL)	Detection method	No. of Salmonellae inoculated 0	10^0	10^1	10^{2c}
Beef (II)	1×10^3	DNAH	−	+	−	+
		BAM	−	+	+	+
Shrimp (I)	6×10^3	DNAH	−	−	+	+
		BAM	−	−	+	+
Shrimp (II)	3×10^3	DNAH	−	+	+	+
		BAM	−	+	+	+
Fish (II)	3×10^5	DNAH	+	+	+	+
		BAM	+	+	+	+
Chicken	1×10^5	DNAH	+	+	+	+
		BAM	+	+	+	+

[a] Experimental conditions were as described in Material & Methods. Approximately 10^0~10^8 salmonellae were inoculated into 1g of homogenized food prior to enrichment.
[b] SPC: Standard plate counts for endogenous bacteria contaminated in the food sample. The figure shown is number of bacteria/mL of food culture. 10X of this figure represents the bacteria numbers/g food sample.
[c] All samples were positive for inoculated salmonellae > 10^2.

Table 4—Detection of Salmonella *in control and inoculated foods with the biotin labeled DNA probe. The CTET→SS (S) culture condition*[a]

Food sample	SPC[b] (CFU/mL)	Detection method	No. of Salmonellae Inoculated 0	10^0	10^{1c}
Shrimp (I)	6×10^3	DNAH	−	−	+
		BAM	−	−	+
Shrimp (II)	3×10^3	DNAH	−	+	+
		BAM	−	+	+
Shrimp (III)	4×10^4	DNAH	−	+	+
		BAM	−	+	+
Fish (II)	3×10^5	DNAH	+	+	+
		BAM	+	+	+
Fish (III)	2×10^3	DNAH	−	+	+
		BAM	−	+	+
Beef (II)	1×10^3	DNAH	−	+	+
		BAM	−	+	+
Pork (I)	2×10^5	DNAH	−	+	+
		BAM	−	+	+
Pork (II)	4×10^4	DNAH	−	+	+
		BAM	−	+	+
Chicken	1×10^5	DNAH	+	+	+
		BAM	+	+	+

[a] Experimental conditions were as described in Table 3 except that the CTET→SS (S) culture condition was used.
[b] SPC: Standard plate counts; See Table 3 for detail.
[c] All samples were positive for inoculated salmonellae > 10^1

2×10^4/g beef or fish. Therefore, to assure growth of salmonellae in these foods, several cultural conditions were investigated for enrichment of salmonellae. We found that when only the preenrichment step followed by filtering the culture through NC membrane, i.e.; the LB (F) culture condition, was used prior to the DNA-DNA hybridization, high contamination levels of salmonellae/g of minced beef before enrichment were required for positive results. Inoculation of 2, 24 or 240. salmonellae to 1g sterilized shrimp in 10 mL of LB would generate 1–3 × 10^9 salmonellae per mL of culture after 18 hr enrichment (unpublished result). Thus, low sensitivity of *Salmonella* detection with LB (F) culture condition could be due to competitive microflora if selective media was not used. When a similar filtration step was performed for bacteria from culture condition of LB→TT→GN or CTET→GN, the initial contamination level of *Salmonella* required to give positive results could be reduced to less than 10^3/g sample. This depended on the type of food sample and the level of naturally contaminated microflora. On the other hand, if the cultural conditions of CTET→SS followed by spotting suspected bacteria on NC membrane, were performed prior to DNA-DNA hybridization, results were sastisfactory. For example, with beef and fish (Tilapia), low numbers of salmonellae (10^0 ~ 10^1/g food sample) could be detected (Fig. 3). Results were clear, unambiguous and coincident with results from the BAM assay.

On evaluation of probe specificity, Salmonella isolates were cultured with LB at 37°C. However, on detection of Salmonella in foods, in order to prevent possible interference from non-Salmonella bacteria, 43°C was suggested for selective culture with TT or CTET broth. We found that using TT or CTET broth as culture media, the Salmonella closely related enterobacteria, such as Citrobacter spp. grew well at 37°C but not at 43°C. Therefore, culture of salmonellae in TT and CTET broth was normally performed at 43°C rather than 37°C.

To further evaluate the culture conditions, i.e. CTET→GN (F) or CTET→SS (S) for specific detection of salmonellae in foods, other food samples purchased from the local outdoor markets were studied. In general, such raw food samples, (shrimp, beef, chicken, fish, etc.) were highly contaminated with populations of competitive microflora. For example, the viable bacterial counts in minced samples of shrimp (I) or chicken were estimated to be 6×10^4 or 1×10^6/g food sample (i.e., 6×10^3 or 1×10^5/mL sample solution) (Table 3,4). These minced food samples, after mixing with CTET broth, were inoculated with salmonellae and subjected to DNA-DNA hybridization according to procedures described for CTET→GN (F) or CTET→SS (S). Results shown in Tables 3 and 4 indicate that although for these food samples, low numbers of salmonella (such as, 10^0 or 10^1/g), could be detected after enrichment, the CTET→ SS(s) procedure was better since results obtained were clear, unambiguous and coincident with BAM results. Some of the hybridization results from the two culture conditions, (e.g., detection of salmonellae in shrimp (II) sample) were shown in Fig. 4.

DISCUSSION

EMSWILER-ROSE et al., (1987) and Flowers et al.,(1987) tried several culture conditions for enrichment of salmonellae in foods. They found that conditions Lac→TT→GN or Lac→SS→GN followed by collecting enriched bacteriaon NC

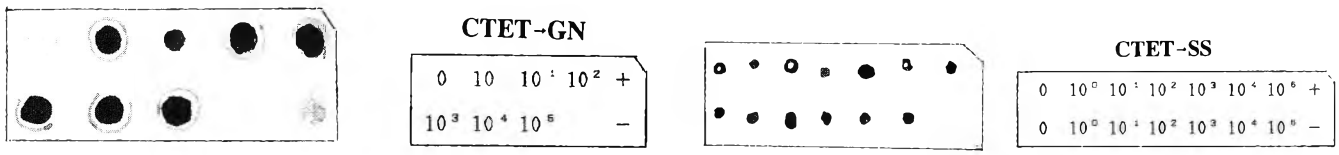

Fig. 4.—Colony hybridization patterns for detection of Salmonella in control and inoculated samples. Experimental conditions were as described in Table 3 and 4, where culture conditions of CTET→GN (F) or CTET→SS (S) was performed prior to colony hybridization with the biotin labeled 1.8 kb DNA probe. The food sample used was shrimp (II) as shown in Table 3 and 4. Bacteria for positive control (+), S. typhimurium*; for negative control, (–),* E. coli.

membrane with filtration could be used prior to DNA-DNA hybridization with ^{32}P-labeled DNA probe. Using such procedures, they could detect low numbers of salmonellae in ground beef, turkey and chicken. However, when similar procedures, such as LB→TT→GN (F) described here were performed prior to DNA-DNA hybridization with biotin labeled probe, we found difficulties in obtaining similar results. A higher contamination level of salmonellae in beef might be required to give positive results. Although differences in filtration apparatus, technique and hybridization sensitivity of DNA probes may account for the difference, such results could also be due to higher contamination levels of endogenous microflora in our beef sample since it was purchased from a conventional outdoor market (Tsen et al., 1989). Other reasons might include short (12 hr) preenrichment and selective enrichment in TT broth.

Although culture conditions of CTET→GN (F) might be used prior to DNA-DNA hybridization, results were not as clear as those obtained from CTET→SS (S) conditions. Using those conditions, low numbers of salmonellae could be detected and hybridization results were clear and unambiguous. This may have been due to the fact that higher numbers of *Salmonella* per hybridization dot were obtained by transferring the *Salmonella* suspected colony from SS plates onto NC membrane. Also 4 hr incubation on LA plate may have been better than 0.2 mL of a 10^{-2} dilution of a GN culture inoculated for 8 hr only. Spotting the suspected bacteria on NC membranes followed by incubation would have made the bacteria colony more condensed.

Recovery of salmonellae from foods by enrichment and selective enrichment is affected by several factors, some are media composition, culture temperature, contamination level of competitive bacteria and the food sample itself (Miller and Koburger, 1984; Andrew W.H. 1987). Thus determining the reasons for different results from different culture conditions is not easy.

Starting from food samples, time required to obtain the final results was about 50 hr for the CTET→GN (F) culture condition and 70 hr for the CTET→SS (S) culture condition. In comparison to the LB→TT→GN (F) and CTET→SS (S) condition, the CTET→GN (F) condition could save one day for bacteria culture. If the filtration step using Bio-dot (Bio-rad) could be improved, better results may be obtained for such CTET→GN (F) procedure. Using ^{32}P labeled DNA probe, Emswiler-Rose et al., (1987) or Flowers et al., (1987), reported that final results for detection of *Salmonella* in ground beef, turkey, etc. could be obtained on day 3 or day 4. If our biotin labeled DNA probe was replaced by the ^{32}P-labeled DNA probe, time required for the detection of *Salmonella* could be shortened since DNA-DNA hybridization process using the biotin labeled probe was usually time consuming.

With biotin labeled DNA probe for detection of *Salmonella* in foods, care should be taken on some hybridization steps. For example, bacteria colonies shouldn't grow too big and hydrolysis of bacteria with proteinase k should be complete. Otherwise, interfering background might be generated. In addition, the quality of NC membrane was important. Difficulties may be found with certain types of nylon membrane. We found that NC membrane (Hybond C-extra) from Amersham was suitable. The major advantage of our method was that large numbers of suspected bacteria could be confirmed on a small piece of NC membrane and no radioisotope was used for any procedures.

Field trials in collaboration with other laboratories may be required to verify the procedures described here. Current results seem to indicate that application of our procedures for detection of *Salmonella* in foods with biotin labeled DNA probe is possible. Similar experiments were performed using biotin labeled oligonucleotide probes and results obtained were also satisfactory.

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Isolation of *Yersinia enterocolitica* and Related Species from Red Meat and Milk

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ABSTRACT

A total of 150 samples, 50 each of beef, lamb and pork from 10 local retail stores in the Brisbane metropolitan area and 50 pasteurized and 150 raw bovine bulk milk tank samples obtained from Queensland United Foods (QUF), were examined for the presence of *Yersinia* spp. over 1 yr. Two isolation protocols were used to recover the organism with subsequent biochemical and serological identification. A total of 114 isolates, consisting of *Y. enterocolitica* (23,12,15,40), *Y. intermedia* (0,3,5,1), *Y. frederiksenii* (4,1,0,10) were obtained from beef, lamb, pork, and milk, respectively. No pasteurized milk samples were positive for the organism. None of the isolated strains was found to harbor the virulence plasmid as indicated by the crystal violet-binding assay.

INTRODUCTION

YERSINIA ENTEROCOLITICA, a major cause of enteric infection (WHO, 1980), is generally recognized as a foodborne pathogen. The organism has been isolated from clinical cases of human diarrhea in several countries (Schiemann, 1989). Many investigators have reported the occurrence of yersiniae in a wide range of substrates including water (Meadows and Snudden, 1982; Schiemann, 1978), milk (Walker and Gilmour, 1986; Vidon and Delmas, 1981) red meat (Nesbakken et al., 1985; Hanna et al., 1976; Inoue and Kurose, 1975) chicken (Norberg, 1981), vegetables (Loiseau-Marolleau and Alonso, 1976) as well as sea food (Peixotto et al., 1979). Reports of sporadic cases and outbreaks of human yersiniosis are amply documented (Simmonds et al., 1987; Tauxe et al., 1987; Anonymous, 1982; Ratnam et al., 1982; Black et al., 1978; Asakawa et al., 1973). In addition, the relationship between pork and human yersiniosis is well established (Lee et al., 1990; Tauxe et al., 1987). The ability of the organism to grow at refrigeration temperatures (Stern and Oblinger, 1980) is another major concern for the food industry. Pasteurization temperature was found adequate to eliminate yersiniae from milk, however, pasteurized milk was reported by some investigators to harbor *Y. enterocolitica* (Walker and Gilmour, 1986), a major outbreak of human yersiniosis was attributed to pasteurized milk (Tacket et al., 1984).

Although occurrence of *Yersinia* species in meat and milk has been investigated in several countries, little has been reported about the incidence of the organism in different foods in Australia. Therefore, it was the aim of our study to check the prevalence of yersiniae in retail red meat packs and in raw and pasteurized milk samples.

MATERIALS & METHODS

Collection of samples

Red Meat. A total of 150 samples, in regular consumer packages, 50 each of beef, lamb and pork (different cuts) were purchased at 10 local retail supermarkets, over 1 yr. Each package was considered 1 separate sample and transferred to the laboratory with a minimum of delay and examined on the day of purchase.

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Milk. A total of 150 raw bovine bulk milk tank samples as well as 50 retail consumer packages of pasteurized milk were obtained from Queensland United Foods (QUF), the major milk processing plant for the city of Brisbane. All milk samples were examined on the same day.

Preparation of samples

About 20g of meat were sampled using sterile scissors and scalpels. Each sample was homogenized in 180 mL of the appropriate enrichment broth in a sterile Stomacher 400 bag for 2 min using a Stomacher 400 (Seward Medical, London). Milk samples (20 mL each) were directly added to the enrichment medium.

Isolation procedures

The protocols proposed by Feeley and Schiemann (1984) and Schiemann (1987) for isolation and identification of yersiniae in food were adopted with a slight modification. A sample portion was added to 180 mL each of Tryptic soy broth (Difco) and preenrichment (PE) broth and incubated at 4°C for 2 wk or 25°C for 2 days respectively. One mL from each enrichment was added to 100 mL of bile-oxalate-sorbose (BOS) broth as secondary enrichment and incubated at 25°C for 5 days. Loopfuls from BOS broth were streaked directly onto cefsulodin-irgasan-novobiocin (CIN) agar (Oxoid, CM653) supplemented by Oxoid antimicrobial supplement (SR 109) and bismuth sulphite (BS) agar (Oxoid CM201) and also after treatment with potassium hydroxide (Auilisio et al., 1980). CIN plates were incubated at 32°C for 18–20 hr and BS plates were incubated at 25°C for 48 hr. One to 3 typical colonies were subcultured on the same 2 media for purification. Presumptive colonies were confirmed as members of the genus Yersinia by heavy inoculation of slopes of Kligler iron agar (KIA) and Christensen's urea agar, both of which were incubated at 35°C for 24 hr. Cultures giving typical reactions on these media were further identified to the species level on the basis of their reactions in the following biochemical tests, incubated at 25°C for 24 h: sucrose, rhamnose, Simmon's citrate, raffinose, melibiose and α-methylglucoside.

Isolates which proved to be *Y. enterocolitica* (sucrose positive and negative for the other 5 reactions) were further biotyped using Wauters biotyping scheme (Wauters et al., 1987). Cultures were then serotyped by H.H. Mollaret and E. Carniel at the Pasteur Institute, Paris. A reference strain (NCTC 10460) serotype 0:3 from the Culture Collection, Department of Microbiology, was used during the isolation procedures as a control positive. This checked efficiency of the protocol in recovering the organism by inoculating enrichment media containing meat or milk samples with 1 mL diluted broth culture containing about 2×10^2-10^3 cells.

Examination of cultures for exoenzyme activity

Isolates of *Y. enterocolitica* were further assessed for pyrazinecarboxylamidase (pyrazinamidase), lipase and lecithinase production. Pyrazinamidase activity was assessed as recommended by Kandolo and Wauters (1985). Lecithinase and lipase production were examined by spot inoculation of test media as follows: Cultures were grown in brain heart infustion (BHI) broth (Difco) and adjusted to give an absorbance reading of 0.9 at 600 nm after which 200 μL were spotted onto nutrient agar plates supplemented with 5% egg yolk emulsion. These were examined after incubation for a white precipitate around the inoculum as an indicator for lecithinase activity. Lipase production was tested using 1% Tween 80 agar plates (Sierra, 1957) on which the formation of a turbid halo around the inoculum indicated lipolytic activity. Both media were incubated at 28°C for 48 hr.

Table 1—Isolation of Yersinia *spp. from red meat and milk*

Source	No. of samples	No. of +ve	Y. entero-colitica	Y. inter-media	Y. freder-iksenii
Beef	50	10 (20)[a]	9 (18)	0 (0)	2 (4)
lamb	50	6 (12)	5 (10)	1 (2)	1 (2)
Pork	50	8 (16)	6 (12)	2 (4)	0 (0)
Raw milk	150	21 (14)	17 (11)	1 (0.7)	5 (3)
Pasteurized milk	50	0 (0)	0 (0)	0 (0)	
Total	350	45 (13)	37 (10)	4 (1)	8 (2)

[a] Parentheses indicate percentage of positive samples.

Crystal violet-binding assay

The ability of *Y. enterocolitica* strains to bind crystal violet (CV), as an indication of the virulence plasmid, was tested as described by Bhaduri et al., 1987.

RESULTS & DISCUSSION

OVERALL RESULTS of the survey are presented in Table 1. *Yersinia* spp., predominantly *Y. enterocolitica*, were prevalent in all kinds of samples except pasteurized milk where the organism was not detected. The isolation rate was variable with the highest percentage in beef (20%) and lowest in lamb (12%) whereas the recovery rate was 16% in raw milk and 14% in pork. While *Y. enterocolitica* was the predominant species, *Y. intermedia* was recovered from 2 pork samples and 1 each of lamb and raw milk. *Y. frederiksenii* was isolated from 2 samples of beef, 1 of lamb and 5 of raw milk. Most positive samples yielded one species, however, 1 sample each of beef and lamb, as well as 2 milk samples yielded *Y. enterocolitica* and either *Y. intermedia* or *Y. frederiksenii*. BS agar was quite inferior to CIN in differentiating typical colonies from background bacteria even with the reference strain used. In addition we did not find postenrichment treatment with potassium hydroxide advantageous with the use of BOS broth as secondary enrichment. Thus, we stopped using both BS agar and alkali treatment after the first 100 samples of our survey. On the other hand, the rapid enrichment in PE broth was negative in 1 sample found positive by cold enrichment. However, it seemed quite promising in shortening the isolation time of the organism. The isolation rate of *Y. enterocolitica* was in agreement with the results of other investigators (Franzin et al., 1984; Moustafa et al., 1983; Christensen, 1981). However, Walker and Gilmour (1986) and Toora et al., (1989) detected the organism in 23 and 24% samples respectively, whereas Fukushima et al., (1984) isolated the organism at levels lower than ours. No isolation was made from pasteurized milk. This was in agreement with the findings of Toora et al., (1989) but in contrast with earlier reports by Hughes (1979) and Tacket et al., (1984). The survey conducted by Greenwood and Hooper (1985) in the UK reported the prevalence of *Y. enterocolitica* during the pasteurizing and bottling processes. The organism was detected in only 1 sample of raw milk suggesting that post-pasteurization contamination was the major source of the orgaism rather than the ability of yersiniae to survive pasteurization. Our isolation rate of yersiniae from red meat was difficult to compare with results from other countries either due to different varieties of samples examined or the absence of comparable published figures. Nesbakken et al., (1985) detected the organism in 30% of pork samples while the incidence was 5% in Japan (Fukushima et al., 1987) with our recovery in between (16%). As pigs are thought to be a major reservoir for *Y. enterocolitica*, more attention is being directed to porcine organs other than meat cuts such as fresh tongues and tonsils where *Y. enterocolitica* was isolated from 90 and 35% respectively (Schiemann and Fleming, 1981). Moreover, it was reported that the frequency of isolation of yersiniae from the tongue and throat of swine was about 10 times greater than that reported for other sites (Schiemann, 1980). In beef, our result was somewhat similar to those reported by Inoue and Kurose (1975) and Leistner et al., (1975) where the organism was recovered from 24 and 16% of samples respectively. However, Stern (1981) and Fukushima et al., (1987) could not detect the organism at all in samples they examined. There are no published reports concerning the isolation rate from lamb as fresh cuts to which we could compare our results.

Table 2—Exoenzyme profile of isolated strains of Y. enterocolitica

Source	No. of isolates	No. of strains producing[a] Li	Le	Py
Beef	23	23	20	23
Lamb	12	12	12	12
Pork	15	13	15	15
Raw mi k	40	9	5	40
Total	90	57	52	90

[a] Abbreviations: Li: Lipase, Le: Lecithinase, Py: Pyrazinamidase.

Table 3—Biotypes and serotypes of isolated strains

Source	Biotype	Serotype[a]
Beef	1A	O:5 (3)
	1A	O:7,13 (9)
	1A	O:4,33 (2)
	1A	O:7,8,19 (3)
	1A	O:7,13,19 (1)
	1A	O:7,13,19-18 (2)
	1A	untypable (3)
	YF[b]	O:3 (3)
	YF[b]	untypable (1)
Lamb	1A	O:4,33 (12)
	YI[c]	O:17 (2)
	YI[c]	O:17-48 (1)
	YF[b]	O:16-16,,29 (1)
Pork	1A	O:7,13 (5)
	1A	O:4,33 (2)
	1A	O:7,13,19 (1)
	1A	O:7,8,19 (3)
	1A	O:7,8,13,19 (1)
	1A	untypable (3)
	YI[c]	O:17 (1)
	YI[c]	O:17 (1)
	YI[c]	O:4,33 (3)
Milk	1A	O:5 (8)
	1A	O:21 (3)
	1A	O:22 (14)
	1A	O:34 (2)
	1A	O:10,34 (13)
	YF[b]	O:16-16,29 (6)
	YF[b]	O:16-16,29-34 (1)
	YF[b]	O:4,33-16-16,29 (2)
	YF[b]	untypable (1)
	YI[c]	O:3 (1)

[a] Parenthese indicate number of strains.
[b] *Y. frederiksenii.*
[c] *Y. intermedia.*

There were slight variations in the biochemical profile of *Y. enterocolitica* recovered in our survey especially for citrate utilization, as most strains isolated from milk were citrate positive (results are not shown). In addition, results concerning lipase and lecithinase were quite surprising (Table 2). Biotype 1 strains are usually positive for both but only 9 and 5 strains from milk were positive for the 2 enzymes respectively. There seems to be a close correlation between the 2 enzymes. However, the large number of milk isolates found negative for lecithinase in particular was in contrast to an earlier report by Wauters (1973) in which all biotype 1 strains were listed lecithinase positive. All isolates were positive for pyrazinamidase and negative for CV-binding assays delineating them from the common pathogenic biovars. An extensive study of different exoenzymes in clinical and food isolates of *Yersinia entercolitica* is being conducted in our laboratory to check the correlation between the bioserovars and different exoenzymes.

Table 3 shows the different serotypes isolated among which 0:5; 0:7,13; 0:22; and 0:10,34 were the predominant serovars. In addition, the 3 strains of 0:21 were found to be salicin and esculin positive while another 4 typical *Y. enterocolitica* se-

rotype 0:3 were obtained. The clinical significance of certain serotypes in our survey especially 0:5 and 0:7,13, although not considered as common pathogens, is well documented. Early reports incriminating these serotypes in both acute and chronic diarrhea with extraintestinal complications (Ratnam et al., 1982; Weissfeld and Sonnenwirth, 1980), have recently been supported by other investigators (Bisset et al., 1990; Jacobs et al., 1989; Simmonds et al., 1987) with subsequent detection of homologous antibodies in patients' sera (Fletcher et al., 1988). In addition, virulent *Y. kristensenii*, a species commonly regarded as nonpathogenic, has been reported by Robins-Browne et al., (1991) to be lethal for mice and yet did not express any identifiable phenotype characteristic of virulent strains of *Y. enterocolitica*. These findings together with the reported cases of biotype 1 *Y. enterocolitica* strains, indicated that the mechanism underlying the pathogenicity of these bacteria differs from those generally associated with commonly pathogenic *Yersinia* species which requires the presence of a 42–48 Mdal plasmid. Bisset et al., (1990) found that a changing and expanding spectrum of *Y. enterocolitica* serogroups was associated with various gastrointestinal and systemic infections. This necessitates careful judgement of isolated strains in clinical laboratories as well as better hygienic standards during food processing.

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Fat Content Effects on Yield, Quality, and Microbiological Characteristics of Chicken Patties

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ABSTRACT

Mixtures of ground chicken thigh meat and fat were formulated to contain 5, 10, 15, and 20% fat. Hunter color values, drip and cooking losses, proximate composition, texture profiles, and rates of microbial spoilage were compared for patties prepared from the mixtures. Raw patties became lighter and more yellow as fat content increased but cooking obscured these trends. As fat content increased, cooking losses and moisture:protein ratios increased. Lower fat patties were harder, springier, less cohesive and chewier than patties containing higher fat levels. Rates of bacterial spoilage were unaffected by fat content.

Key Words: Chicken, patties, microbiology, fat, texture

INTRODUCTION

USDA regulations limit the fat content of raw products labeled as "ground beef" or "hamburger" to 30% but no similar regulations apply to ground poultry products. Instead, the composition must be "in natural proportions" (USDA, 1970), leaving the meaning of that phrase open to question. USDA Handbook #8 (USDA, 1979) provides no data on the composition of ground poultry. No data are available concerning the composition of products currently marketed, but products of commerce are often advertised as "low fat" to exploit the public's perception of poultry meat as "healthful." Since consumer consciousness of the role of diet in maintaining good health is increasing (Erdman, 1989), it seems likely that consumer demand will increase for more specific standards regarding the fat content of ground poultry meat. However, the effect of percentage fat content on yield and quality of ground poultry is unknown. Cross et al. (1980) found that with ground beef, increasing the fat content from 16 to 28% resulted in increased tenderness and juiciness. However, when the patties were cooked, increasing the fat also increased fat losses and decreased moisture losses. Overall cooking lossess were unaffected by fat content. In order to establish rational standards for ground poultry, data are needed which show the effects of fat content on product quality, yield, and storage characteristics. The objective of our study was to evaluate the effects of variation in fat content on yield, quality, and storage characteristics of ground chicken thigh meat patties.

MATERIALS & METHODS

Preparation of samples

Ingredients for the patties were commercially prepared, hand-deboned chicken thigh meat and abdominal fat. Skin was not included in the formulations. Both were procured from a local processor immediately after carcass chilling. Ingredients were transported to a nearby pilot facility where they were chilled to 2°C in a −30°C freezer (about 45 min). The ingredients were unrefrigerated during transit (about 30 min). Each product was ground separately through a 15.9 mm plate and then sampled for moisture, fat, and protein analysis. After sampling, the ground ingredients were stored at −30°C for three days. Prior to preparing the patties, the meat and fat were tempered at 5°C until they reached 2–3°C.

Batches (600g each) of meat and fat were formulated to contain 5, 10, 15, or 20% fat. Each batch was individually prepared by regrinding the required amount of thigh meat and abdominal fat together through a 9.5 mm plate and hand mixing the combined ingredients in a plastic bag for 3 min. All processing equipment was washed with detergent and water between batches and operators wore clean rubber gloves to minimize bacterial contamination. Patties were formed from 100g aliquots of each mixture using sterile 15 × 100 mm petri dishes as molds. The patties were tempered for 30 min at −30°C to expedite removal from the petri dishes, weighed, and then individually packaged on plastic foam trays with polyvinyl chloride film overwrap. Patties for yield and quality studies were stored at −30°C, and those for microbiological analysis were stored at 4°C.

Yield and quality evaluation

Five batches of meat and fat were prepared for each fat level. Four patties were prepared from each batch. Thus, yield and quality were determined on 20 patties from each fat level. The experiment was replicated with a second procurement of thigh meat and abdominal fat for a total of 40 patties for each fat level.

Drip and cooking losses

After 7 days frozen storage, the pattie were thawed for 24 hr at 4°C, drained on paper towels, and weighed. Drip loss was calculated by difference and expressed as a percentage of initial weight. Each patty was cooked on an electric grill set at 176°C to an internal endpoint of 73°C as determined by a probe inserted into the center of each patty. They were turned frequently during cooking to ensure uniform heating, then cooled to room temperature and weighed. Cooking loss was calculated as percentage of the weight immediately before cooking.

Hunter color values

Hunter color values for lightness (L), redness (a), and yellowness (b) were measured on the surface of each patty after thawing, on the surface after cooking, and on a cut surface after cooking (internal) using a Minolta Chroma Meter CR-200b (Minolta Camera Co., Ltd., 30, 2-chrom, Azuchi-Machi, Higashi-Ku, Osaka 541, Japan).

Objective texture profile analysis

Objective Texture Profile analysis was performed in accordance with Lyon et al. (1980). Duplicate 2.5 cm cores were cut from each patty. They were compressed twice to 60% of initial patty thickness between a fixed steel plate and a movable steel plate, attached to a 100 kg compression load cell on an Instron Universal Testing Machine

Table 1—Fat content effects on some physical factors of chicken thigh meat patties

Fat content	N	Drip loss %	Cooking loss %	H_2O:Protein
5	40	0.44[a]	32.2[b]	2.71[c]
10	40	0.21[a]	33.4[b]	2.77[b]
15	40	0.48[a]	35.9[a]	2.82[a]
20	40	0.33[a]	35.4[a]	2.80[a]
Mean	160	0.37	34.2	2.77
Std. Dev.		1.03	6.4	0.91

[a-c] Values in the same column which share no common superscripts are significantly different ($P<0.05$).

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Table 2—Effect of fat content on Hunter color values[d] of raw ground chicken thigh meat and surfaces and interiors of grilled patties

Fat content	N	Raw			Grilled surface			Grilled interior		
		L	a	b	L	a	b	L	a	b
5	40	46.5[c]	10.0[a]	18.6[c]	51.5[b]	9.6[ab]	24.0[b]	48.4[c]	6.6[b]	18.1[b]
10	40	47.3[c]	10.2[a]	20.1[b]	52.4[ab]	9.9[a]	25.7[a]	48.5[c]	7.1[a]	18.6[ab]
15	40	50.6[b]	10.3[a]	20.4[a]	52.4[ab]	8.6[b]	24.6[a]	48.9[b]	6.5[b]	18.2[b]
20	40	54.2[a]	10.0[a]	21.6[a]	54.1[ab]	9.1[ab]	25.6[a]	53.7[a]	6.4[b]	18.9[a]
Mean	160	49.4	10.1	20.1	52.5	9.3	24.9	49.7	6.6	18.4
Std. Dev.		12.6	2.1	3.0	6.9	2.3	2.7	19.5	1.9	2.5

[a-c] Values in the same column which share no common superscripts are significantly different ($P < 0.05$).
[d] Hunter L = lightness, a = redness, b = yellowness.

Table 3—Effect of fat content on objective texture profile attributes of grilled chicken thigh meat patties

Fat content %	N	Hardness kg	Springiness cm	Cohesiveness	Chewiness kgcm
5	40	4.2[a]	0.49[a]	0.40[a]	0.74[a]
10	40	4.1[ab]	0.49[a]	0.37[b]	0.71[a]
15	40	3.9[b]	0.45[b]	0.34[c]	0.56[b]
20	40	3.9[b]	0.38[c]	0.34[c]	0.47[b]
Mean	160	3.9	0.46	0.37	0.63
Std. Dev.		1.2	0.25	0.13	0.39

[a-c] Values in the same column which share no common superscripts are significantly different ($P < 0.05$).

(Instron Corp. Canton, MA). The Instron was set at 20 kg full scale load with cross head speed 50 mm/min. Values for hardness, springiness, cohesiveness, and chewiness were measured as described by Lyon et al. (1980).

Chemical analysis

The raw ingredients were analyzed in duplicate for protein (macro-Kjeldahl), fat (soxhlet extraction with diethyl ether), and moisture (vacuum-oven drying) and the cooked patties for moisture and protein according to AOAC (1980).

Microbiological analysis

Shelf life of the patties was determined as follows: 4 batches of meat and fat for each fat level were prepared as described. Six patties were prepared from each batch. One patty from each batch was stored at 4°C for 0, 3, 6, 9, 12, or 15 days. Thus, a total of 4 patties for each fat content, representing all batches were placed under each storage condition. Before conducting the microbiological analyses, the patties were smelled by an untrained analyst for presence of off-odor. The microbiological analyses were conducted by suspending a 1g sample from each patty in 10 mL of 1% peptone solution (Difco, Detroit MI), then stomaching the suspension (Colworth Stomacher 80, Takmar Co., Cincinnati, OH) for 1 min. Serial dilutions were prepared and the psychrotrophic bacteria enumerated using a pour-plate technique in duplicate. The growth medium was plate count agar (Difco, Detroit, MI) and the plates were incubated at room temperature (21–23°C) for 72 to 96 hr (APHA, 1966). The plates were manually counted and the $\log_{10}$ of the average of duplicates was used for statistical analysis. The experiment was replicated with a second batch of meat and abdominal fat for a total of 8 patties for each fat level and time of storage combination.

Statistical analysis

The data were analyzed by analysis of variance using batches within replicates × fat levels as the error term. Means for fat levels were separated at $P \leq 0.05$ using Duncan's Multiple Range Test (Steel and Torrie, 1980) and the SAS GLM procedure (SAS Institute, Inc., 1987).

RESULTS & DISCUSSION

Drip loss, cooking loss, and composition

The overall mean for drip loss was 0.37% and did not differ significantly among fat levels (Table 1). Cooking loss was greater for patties containing 15 and 20% fat than for those containing lesser amounts. This trend was expected since more fat would likely be lost from patties containing high levels of fat than from those containing lower levels (Hunt et al., 1990). However, the moisture:protein ratio of the cooked patties increased as fat content increased. Assuming that little protein was lost when the patties were cooked, increasing the fat in the patties apparently reduced moisture loss. We offer no obvious explanation why increasing fat content of raw chicken patties should decrease moisture loss upon cooking, but a similar trend was observed by Cross et al. (1980) and Hunt et al. (1990) with beef patties. Perhaps some of the fat and moisture became entrapped in an emulsion during grinding and more moisture was retained during cooking. The data indicate that minimum cooking loss was achieved if the raw product contained 10 to 15% fat. However, higher levels of fat might result in a more moist final product.

Hunter color values

Color of the patties containing 20% fat was significantly lighter (higher L value) than that of those with 15% fat. Pattie with 15% fat were significantly lighter than those with 5 or 10% fat (Table 2). There was no significant difference in lightness between patties containing 5 and 10% fat. Those containing 15 or 20% fat were also more yellow (greater b value) than those with lower fat. For raw patties, no significant differences in redness (a value) were observed between treatments. Grilling tended to obscure these trends somewhat on both surface and interior color values. However, for the cooked patties, the 20% fat product was lighter and more yellow than the 5% fat products. Redness scores remained similar regardless of fat content.

Objective texture profile analysis

As fat content increased, hardness, springiness, and cohesiveness also increased (Table 3). Therefore, since chewiness is the product of the other three textural attributes, it also increased (Table 3). Even though Lyon et al. (1980) have shown that the objective texture profile analysis highly correlated with taste panelists perceptions of texture of chicken patties, the optimum degree of these textural properties for consumer preference is unknown. The ideal combination may vary with the way the product is used in the home or foodservice establishment. For this reason, it seems unlikely that a single ideal standard for fat content in terms of textural attributes in the cooked product can be established. Nevertheless, both consumers and food processors need to know how texture varies with composition.

Microbiological analysis

The psychrotrophic bacterial growth curves were typical of those normally found on processed poultry products (data not shown, Ayers *et al.*, 1950). Bacterial spoilage (off-odor and bacterial counts > 10^7 CFU/g) occurred after about 6 days of storage at 4°C and was unaffected by fat content. These data indicated that shelf life for ground chicken was unaffected by fat content.

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Sodium Tripolyphosphate and Sodium Ascorbate Monophosphate as Inhibitors of Off-flavor Development in Cooked, Vacuum-packaged, Frozen Turkey

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ABSTRACT

Sodium tripolyphosphate (STP) or sodium ascorbate monophosphate (SAsMP) in water solutions (0.3 and 0.5% levels) or water only were added to ground turkey which was cooked, vacuum packaged, and stored frozen. Soapy flavor was higher, but rancid flavor and hexanal and bathophenathroline-chelateable (nonheme) iron contents were lower in samples with phosphate salts. Samples without phosphates contained the greatest amount of bathophenathroline-chelateable iron; samples with 0.5% STP contained the least. The addition of phosphate salts decreased cooking losses and increased moisture but did not affect the fat content. Generally, intensity scores for stale and rancid aroma and flavor attributes were low, <1 for all samples.

Key Words: turkey, off-flavor, ascorbate-monophosphate, nonheme-iron, hexenal, oxidation

INTRODUCTION

THE MARKET for precooked meat has grown to meet the consumer demand for convenient, ready-to-eat, food products. "Fresh" flavor quality is necessary for consumer acceptance. Work recently completed in our laboratory indicated that vacuum packaging significantly reduced "warmed-over" and rancid flavors and retained the meaty flavor of cooked, frozen and stored beef (Hwang et al., 1990). Extensive research on development and causes of "warmed-over" flavor has been published and reviewed. Recent reviews are: Lillard (1987); Melton (1986); National Live Stock and Meat Board (1988); Peng (1986); St. Angelo et al. (1987); and St. Angelo and Bailey (1987).

As early as 1958, the contribution of lipid oxidation to off-flavor in meat was suggested. Tims and Watts (1958) studied the antioxidant effects of phosphates and ascorbic acid separately or combined and found that, at some levels, these additives had a synergistic action and reduced the prooxidant action of iron in meat. Sato and Hegarty (1971) also studied this relationship and concluded that phosphates reduced prooxidant activity in cooked beef as measured by TBA values. More recently, Roozen (1987) studied several antioxidants, including tripolyphosphate, ascorbate, and ascorbate-phosphate salts, in relation to phospholipid oxidation. He reported lower TBARS (thiobarbituric acid reactive substances, measured as absorbance readings at 532 nm) for samples containing 5000 ppm of the ascorbate-phosphate salts than for control samples. St. Angelo et al. (1988) reported flavor (brothy, painty, cardboardy) notes and hexanal content of cooked ground beef with ascorbate-2-phosphate (Mg salt) stored for 3 days at 4°C. Painty and cardboardy flavors and hexanal content were reduced in samples containing the ascorbate phosphate salt. Although the use of chemical (St. Angelo et al., 1988) and natural (Rhee, 1987) compounds is effective in preventing stale, off-flavor in stored cooked meat, one problem encountered with their use may be the flavor imparted by the particular added compound.

Both vacuum packaging and tripolyphosphate salts have reduced stale ("warmed over") flavor development in stored cooked meats. The effectiveness of various phosphate salts in vacuumed-packaged cooked meats has not been studied. The purpose of our current study was to determine the effect of different levels (0.3 and 0.5%) of two sodium phosphate salts (sodium tripolyphosphate and sodium ascorbate monophosphate) on hexanal and bathophenathroline-chelateable (nonheme) iron contents and flavor and aroma components of cooked, vacuum packaged, frozen ground turkey.

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MATERIALS & METHODS

Materials

Ground turkey meat was purchased from a local supermarket on each of 4 days, 20 hr prior to cooking. Additions to the ground turkey were 5% (by weight) water (reverse osmosis water) or solutions of sodium tripolyphosphate (Stauffer Chemical Co., Washington, STP) or sodium L-ascorbate 2-monophosphate (SAsMP) that had been prepared from the magnesium salt (Showa Denko, Tokyo, Japan) by ion-exchange chromatography (Liao and Seib, 1990). A stock solution of 10% by weight as disodium L-ascorbate 2-triphosphate was prepared. The concentration of SAsMP was determined by dilution in a 0.1M carbonate buffer (pH 10) using ascorbance at 258 nm and molar extinction of 16,000 L/mole × cm.

Sample preparation

Phosphate salt levels of 0.3 and 0.5% were tested; ground turkey was placed in a Kitchen-Aid® mixer (Model K-45 Hobart Manufacturing Co.) with 5% water or solution of dissolved salts and mixed for 15 sec. Two 200-g patties were formed for each experimental unit using a 9-cm diameter form (Tupperware®). Two patties were placed in a Reynolds® Oven Cooking Bag (Reynolds Metals Co.), refrigerated for 1 hr to allow tempering of solutions and ground muscle. Bagged patties were placed on wire racks 10 cm high on an aluminum broiler pan. Ten cuts were made through both layers of the bag around each patty. The patties were cooked in a rotary hearth oven at 162°C (325°F) to an internal temperature of 80°C, removed from the oven, and weighed. Total cooking losses were determined and reported as a percentage based on weights before and after cooking. Patties were cooled for 15 min and then vacuum packaged (two patties per bag) in polyethylene-polyvinyl chloride laminated bags (Koch Supplies, Kansas City, MO; 23 × 30.5 cm (8 × 12 in.). A Hollymatic vacuum packager (Hollymatic Corp.) was used with a vacuum time setting of 5 and sealing time setting of 2. Samples were frozen at −14°C and stored frozen for 5 mos.

Vacuum packaged samples were removed from the freezer, four 1-cm cuts were made in the top of the package, and patties were placed on a Microware® rack (Anchor Hocking Corp.) 29.8 × 26.0 cm (8 × 12 in.) and heated for 4 min in a microwave oven (Amana Radarange®, Model RR-70A 1500 watts, Amana Refrigeration, Inc.). After 2 min, the bags were turned over and the ends were reversed. Patties were heated an additional 2 min, removed from the oven, and served to the panelists. One of the patties was cut horizontally, and each half was cut into six wedges; two wedges were served to each panelist. A patty (frozen raw on the initial day of storage) was thawed, cooked and served as a reference sample. Treatments were randomized for order of heating and serving.

Table 1—Measurements for cooked, vacuum packaged, turkey patties with and without phosphate salts, stored frozen for 5 months

		STP		SAsMP	
Measurement	0	0.3%	0.5%	0.3%	0.5%
Cooking time, min	48.0	48.2	48.4	48.8	47.2
Cooking loss,	37.1[a]	34.3[b]	30.7[c]	35.0[b]	34.0[b]
pH	6.3[d]	6.6[bc]	6.9[ab]	6.6[c]	6.7[a]
Moisture, %	58.2[b]	60.6[a]	61.8[a]	60.2[a]	60.2[a]
Fat, %	10.4	9.8	9.7	10.3	9.9

[abcd] Means with different letters are significantly different ($p < 0.05$).

Sensory analysis

A professional five-member taste panel (Kansas State University Sensory Center, panelist had a minimum of 180 hr of training and 300 hr of experience) evaluated the samples using a 15 cm line anchored at both ends (0-none to 15-intense). The descriptors used were Aroma: meaty/turkey, stale (warmed-over), rancid (painty); Flavor: meaty/turkey, stale (warmed-over), rancid (painty), bitter, metallic, and soapy; and Aftertaste: soapy and metallic. These attributes were selected based on previous work (unpublished) establishing flavor profiles of cooked ground turkey with and without phosphate salt. Seven panelists were trained and five were selected to serve on the panel based on their precision in analyzing the flavor and aroma components. At each of four evaluation periods, freshly cooked ground turkey was served as a reference sample. Average intensity scores of attributes for the reference sample were: Aroma: meaty (turkey) = 9.1, stale (warmed-over) <0.1, rancid (painty) <0.1, for Flavor: meaty (turkey) = 8.6, stale (warmed-over) = 0.2, rancid (painty) <0.1, bitter = 0.2, metallic = 1.2, and soapy < .1; and for Aftertaste: soapy = 0.1 and metallic = 1.4. The samples were served warm in preheated glass cups. Water, unsalted-surface crackers, and apples were used to cleanse the palate between samples.

Chemical analyses

The second patty was pulverized and pH, moisture, fat, hexanal, and iron contents were determined.

pH, moisture, and fat. pH measurements were made for slurries of 10-g meat and 90 mL water with a Corning pH meter (Model 140). Moisture was determined by AOAC (1984) method, and fat was determined by the method of Folch et al. (1957).

Hexanal and iron. Because hexanal develops very rapidly at room temperature, all of the samples were vacuum packaged immediately after reheating until time of distillation (within 2 hr). The order of distillation was the same random order for preparation and evaluation by the panel. The headspace method of Lin and Jeon (1985) was used to determine hexanal content. At the time of distillation, one-half patty was pulverized in a food processor (West Bend Co.™ Model 6500) for 30 sec and a 2g sample plus 1 mL (10 μg) of 4-heptanone (Sigma H-5387) as an internal standard were placed in a Kemmerer Hallet distillation unit. The distillates were analyzed for hexanal on a Shimadzu GC-9AM Gas Chromatograph with a Supelco Carbowax capillary column (30 × 0.75m and 1.2 mdf). Response was recorded on an Altex Model C-RIA integrator, and the ratio between the areas of hexanal and the internal standard were determined. Calculations were made from a response curve that was linear from 0.5 to 40 μg hexanal. Additional determinations of hexanal contents were made for these samples after they were stored frozen for 6 wk in nonvacuum packaging. The method of Schricker et al. (1982) for "nonheme iron" was used to determine bathophenanthroline chelateable (BPC) iron for 5-g duplicate samples.

Experimental design and data analysis

Data were subjected to analysis of variance using a complete block design with replications (or blocks) being evaluation periods. There were four replications (evaluation periods) of each treatment. Panelist was included as a source of variation. Least significant differences were calculated to indicate differences among means.

RESULTS & DISCUSSION

FOR COOKED SAMPLES vacuum packaged and then stored frozen for 5 mo, the addition of sodium phosphate salts significantly increased pH and moisture content (Table 1). Cooking losses were reduced from 37% (without phosphate salt) to 34–35% with 0.3% STP and SAsMP (0.3 and 0.5%) and to 31% with 0.5% STP. Polyphosphates generally behave as bases in meat systems (Prusa and Bowers, 1984). Higher pH for muscle tissues containing STP has been reported previously. Information is limited about the use of the ascorbate 2-monophosphate compound in meat systems. However, the pH of a water solution (0.5%) of SAsMP was 7.8 and that of STP, 8.9 (higher than meat) so a slight increase in pH of the meat was expected. Fat content was about 10% and unaffected by treatment.

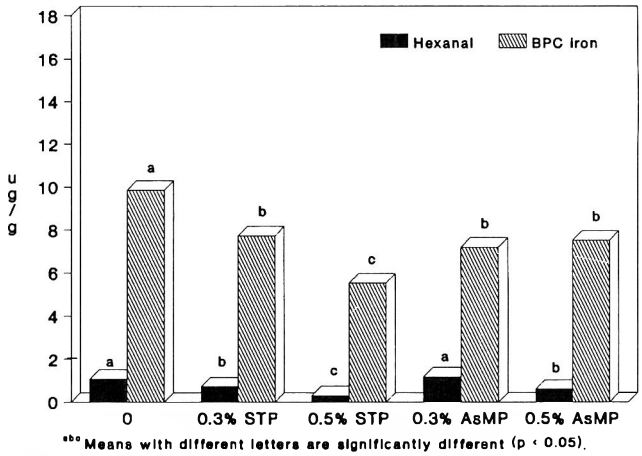

Fig. 1—Hexanal and bathophenanthroline-chelateable (BPC) iron contents of cooked vacuum packaged ground turkey stored frozen for 5 mo.

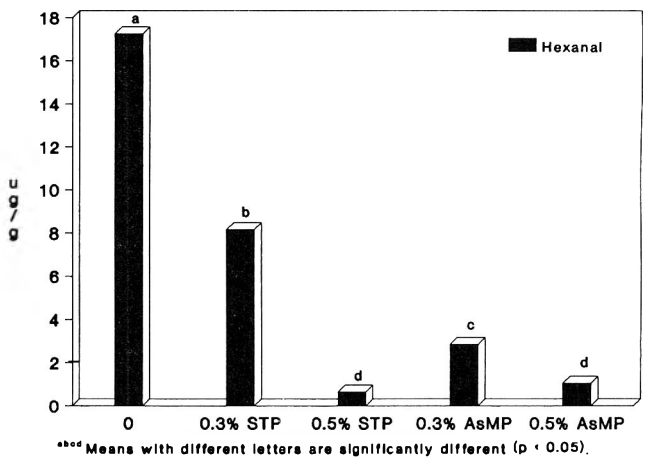

Fig. 2—Hexanal content of ground turkey after additional 6 wk frozen storage in nonvacuum package.

Hexanal content was significantly lower for samples with STP than for those with SAsMP and lower for those with 0.5% levels of salts than with 0.3% (Fig. 1). Samples with no salts and those containing 0.3% SAsMP were similar in hexanal content—1.08 μg/g and 1.18 μg/g, respectively. In general, the hexanal values for the vacuum packaged stored turkey were low, only slightly higher than we found for freshly cooked ground turkey, 0.8–1.0 μg/g. We wanted to determine whether hexanal values would change if meat samples were not protected from oxygen by vacuum packaging. Earlier work in our laboratory had indicated a 50–100% decrease (Hwang et al., 1990) in hexanal content if cooked muscle were stored in vacuum packaging.) Therefore we stored the remaining pulverized samples for an additional time in Ziploc™ bags (Dow Chemical, 2.7 mil) but not vacuum-packaged. When those samples

Table 2—Aroma and flavor of cooked, vacuum-packaged, reheated, turkey patties with and without phosphate salts, stored frozen for 5 months

		STP		SAsMP	
Measurement	0	0.3%	0.5%	0.3%	0.5%
Aroma					
Turkey	4.8^{ab}	5.4^{a}	5.2^{a}	5.5^{a}	3.7^{b}
Stale	0.8	0.4	0.3	0.5	0.9
Rancid	0.2	0.1	0.2	0.1	0.3
Flavor					
Turkey	4.3^{cd}	5.0^{ab}	4.7^{bc}	5.3^{a}	4.1^{d}
Stale	0.9	0.8	0.6	0.7	0.8
Rancid	0.4^{a}	0.1^{b}	0.1^{b}	0.1^{b}	0.1^{b}
Bitter	0.4^{a}	0.3^{ab}	0.1^{c}	0.1^{c}	0.1^{bc}
Metallic	1.5	1.3	1.3	1.2	1.6
Soapy	1.4^{c}	1.5^{bc}	2.8^{a}	1.1^{c}	2.3^{ab}
Aftertaste					
Soapy	1.3	1.7	2.4	1.5	2.2
Metallic	2.4	2.2	2.2	2.1	2.8

^{abcd} Means with different letters are significantly different ($p < 0.05$).

were stored frozen for an additional 6 wk in nonvacuum packaging, the hexanal content of the samples without phosphate salts increased sharply (Fig. 2). The difference in hexanal content among the treatments was much greater after storage. Those containing the higher levels of the salts had the lowest values. The hexanal content of the samples containing 0.3% STP was the highest of those containing salt, but the sample with no salt contained more than twice as much hexanal as that sample.

Iron (BPC iron) was significantly reduced by the addition of all phosphate salts—from 9.9 μg/g for those without to 7.2–7.7 μg/g for samples with SAsMP and 0.3% STP and to 5.5 μg/g for samples with 0.5% STP (Fig. 1). The function of "nonheme" iron as a catalyst responsible for oxidation of cooked meats has been reported by several researchers (Igene et al., 1979; Love, 1983; Rhee et al., 1987). Tims and Watts (1958) suggested that phosphate salts prevented autoxidation by chelating heavy metal ions. This could be an explanation for the decreased hexanal content and rancid flavor of phosphate-treated samples. Though our results indicated that there was less BPC iron in samples containing phosphates salts, the mechanism by which this occurred was not clear. Complexation of the iron by the phosphate salts as suggested by Tims and Watts (1958) is a possible explanation. Another theory could relate to the stabilization of the heme pigments to reduce release of iron. The phosphate salts we used increased the pH of the meat and Trout (1989) reported that high pH decreased myoglobin denaturation in meat. For our samples, there may have been some mechanism by which the phosphate salts stabilized the porphyrin ring and prevented release of iron during cooking. Further study of the mechanism by which phosphate salts influence "free" iron is underway.

Turkey flavor and aroma were significantly more intense for samples containing the lower level (0.3%) than the higher level (0.5%) of SAsMP. Differences between the 2 levels of STP were not significant, but followed the same trend (Table 2). The intensity of turkey flavor and aroma components of the experimental samples were considerably lower than the reference sample. All experimental samples were cooked, frozen, stored, and reheated and the reference sample was freshly cooked. Loss of meaty character usually precedes development of stale and rancid flavor.

The stale ("warmed-over") aroma and flavor components were not affected by treatment. Flavor of samples with 0.5% levels of both phosphate salts was significantly more soapy than that of samples without or with lower levels of the salts. Aftertaste intensity scores for "metallic" and "soapy" tended to be higher than the initial scores.

Even though the intensity of the rancid (painty) characteristic was lower for patties with the phosphate salt additives than for those without, all scores were very low (<0.5). The flavor and aroma components related to lipid oxidation were of low intensity (<1) for all samples—even those with no phosphate salts. Earlier work in our laboratory (Nolan et al., 1989) demonstrated that vacuum packaging significantly reduced stale and rancid flavor and aromas. Had samples been non-vacuum packaged, greater differences might have been found.

The ascorbate phosphate compound is not an approved food additive, so testing has been limited in food products. St. Angelo et. al. (1988) used the Mg form of the salt and found "painty" and "cardboardy" flavors and hexanal content were reduced. Our preliminary work with the magnesium salt indicated off-flavor problems, so we used the sodium form. Sato and Hegarty (1971) used sodium ascorbate (0.5%) in ground beef and reported reduced TBA values. High levels of ascorbic acid (1,000 to 10,000 ppm) inhibited whereas lower levels (3 to 500 ppm) increased warmed-over odor of a muscle-tissue water-extract model system. They suggested that ascorbic acid functioned by keeping part of the iron in the ferrous state or acting as an oxygen scavenger.

CONCLUSIONS

BOTH tripolyphosphate and ascorbate monophosphate salts provided slight additional reduction in lipid oxidation and stale or rancid off-flavor development for vacuum-packaged, cooked, ground turkey. However, more intense soapy off-flavor was present in the sample with 0.5% levels. Generally, stale/warmed-over flavor and rancid/painty flavor and aroma scores and hexanal contents were low for all cooked vacuum-packaged, frozen samples. This probably was attributable to vacuum packaging. When samples were subjected to further frozen storage in nonvacuum packaging, differences in hexanal content among treatments were magnified and those containing no salt had 2 to 17 times more hexanal than any sample containing a phosphate salt.

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Ionic Strength and pH Effects on Composition and Microstructure of Yolk Granules

D. CAUSERET, E. MATRINGE, and D. LORIENT

ABSTRACT

We investigated the microstructure of hen egg granules. Polyacrylamide gel electrophoresis revealed that the granules consisted of three proteins (low-density lipoprotein, lipovitellin and phosvitin). Atomic absorption spectrometry revealed a notable amount of divalent and trivalent cations. Scanning electron microscopy showed that pH modifications induced the loss of granule structure by solubilization of proteins. Similar observations were made when ionic strength was increased by addition of sodium chloride. All results were indicative of the existence of phosphocalcic bridges between phosphate groups of the three proteins.

Key Words: Egg yolk, microstructure, granules, phosphocalcic-bridge, pH

INTRODUCTION

EGG YOLK is a complex mixture of different microparticles held in suspension: spheres, granules, profiles. The spheres, minor components, were first observed under optical microscopy by Moran (1925). Romanoff and Romanoff (1949) determined their diameter, which ranged between 4 and 150 μm. They contain lipoprotein or lipid droplets (Grodzinsky, 1951; Bellairs, 1961). Bellairs (1961) and Chang et al. (1977) studied the profiles, made of low-density lipoproteins (LDL). Under an electron microscope their diameter is about 0.025μm. The granules are the major components of yolk. According to Burley and Cook (1961), they contain about 70% lipovitellins, 16% phosvitins and 12% low-density lipoprotein (LDL). Their size ranges from 0.2 to 2μm (Bellairs, 1961; Chang et al., 1977). Burley and Cook (1961) observed their dissociation under high-saline conditions (1.71 M NaCl).

Radomski and Cook (1964) suggested that the granular unit consisted of a lipovitellin-phosvitin complex and lipoprotein. These proteins are well characterized. Lipovitellin is a mixture of two components, α and β (Bernardi and Cook, 1960), which differ by their phosphorus content. Their molar mass is about 400,000g and their lipid content is between 16 and 22%. Phosvitin is a phosphoglycoprotein containing 10% phosphorus and 5 to 6% glucidic residues. Connelly and Taborsky (1961) isolated two components, α and β. The β-phosvitin contained 9.20% phosphorus, while α-phosvitin contained 2.97% (Abe et al., 1982). The molar mass ranged from 35,000 to 40,000g (Mok et al., 1961; Cook, 1968). Low-density lipoproteins have a micelle-like structure and consist of about 89% lipids, 3% glucides (Nichols et al., 1954). LDL_1 and LDL_2 differ by their molar mass (10.3×10^6g and 3.3×10^6g, respectively).

Although the protein composition of yolk is well known, only a few results deal with interactions between these proteins. We found no published reports since about 1980 in this field. The aims of our study were to provide further information on interactions which contribute to maintain the granular structure and to investigate the importance of the modification of pH, ionic strength and nature of mineral cations on some physicochemical properties of the yolk, i.e., solubility and viscosity.

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MATERIALS & METHODS

Preparation of granules and pH adjustment

Eggs of Warren Isa hens, less than 48 hr old were used. The yolks were separated from the albumen and rolled on absorbing-paper to remove adhering albumen and chalazas. The vitellin membrane was broken and the vitellus was immediately collected. Yolk was diluted with an equal volume of aqueous NaCl solution (1% w/v) and the resulting mixture was centrifuged at $10,000 \times g$ for 45 min, according to the method of McBee and Cotterill (1979). The sedimented granules were washed with deionized water and centrifuged once more. The pH of each suspension was adjusted by adding 1M HCl or NaOH solution with continuous stirring.

Analytical analyses

The protein content was determined by the Kjeldahl method. The lipid content and dry weight were estimated by AOAC (1980) methods. The contents of calcium, magnesium and iron of yolk were obtained by atomic absorption spectrophotometry. For calcium and magnesium, a solution of lanthanum was used as a spectrophotometric buffer (Pinta, 1979). The dilutions were prepared with deionized water.

Polyacrylamide gel electrophoresis

Proteins were separated by polyacrylamide gel electrophoresis in a vertical slab unit according to the method of Maurer (1971). In order to allow the migration of proteins in the polyacrylamide gel, it was necessary to dissociate the granules in 0.6 M aqueous NaCl solution (for a solution of granules at 2% proteins). Electrophoretic conditions were: stacking gel–4% polyacrylamide (T=3.85%, C=2.6%); running gel–7.5% polyacrylamide; electrophoresis buffer–Tris 0.05M/Gly 0.04M, pH 8.3. Bromophenol blue was the tracking dye used to monitor progress of the protein separation. The gels were fixed in a solution of trichloroacetic acid (12%) for 1 hr. The staining solution was 0.001% Coomassie blue in a solution of 0.1M aluminum nitrate/25% isopropanol/10% acetic acid/1% Triton X-100, prepared by the method of Hegenauer et al. (1977). The destaining solution was acetic acid/methanol/water (70:300:630, v/v/v).

Scanning electron microscopy

The microstructure of the yolk granules was observed in a Jeol-JSM 35 scanning electron microscope, with cryo-unit. All samples were frozen and fractured in liquid nitrogen, then immediately transferred into the vacuum space of the cryo-unit. The accelerating voltage was not allowed to exceed 8 kV to prevent local overheating of the sample.

Table 1–Composition of granular fractions. Average of five replications. Values in parentheses from Burley and Cook (1961)

	Concentration (mg/g)	Percentage of yolk
Dry weight	447 ± 1 (500)	25.3 (23)
Proteins	265 ± 8 (–)	47.8 (–)
Lipids	148 ± 3 (150)	13.4 (10)
Calcium	5.3 ± 0.10	81.0 (70)
Magnesium	0.32 ± 0.01 (–)	71.0 (–)
Iron	0.17 ± 0.01 (0.14)	98.0 (95)

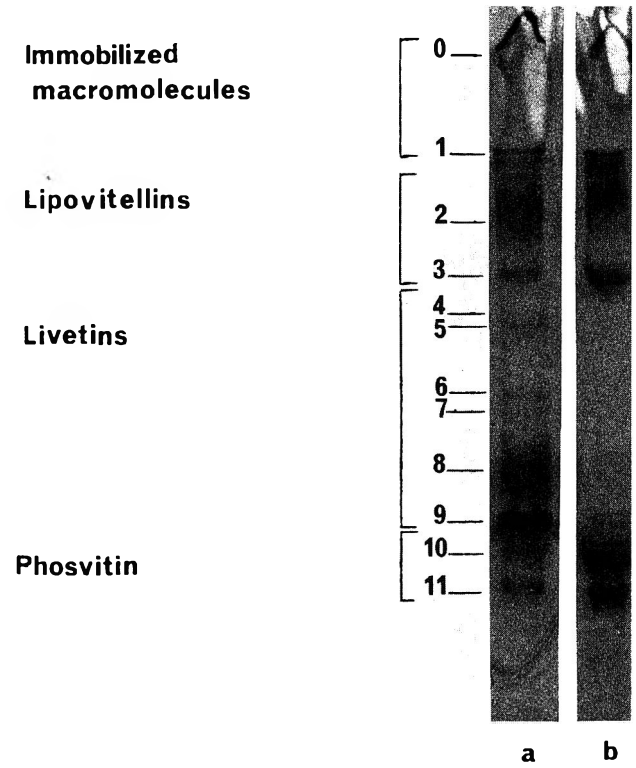


Fig. 1—Polyacrylamide gel electrophoretic patterns of (a) yolk and (b) granules.

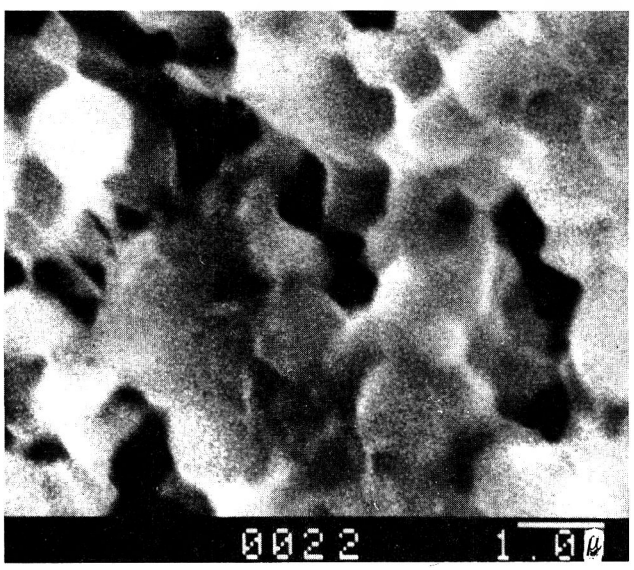


Fig. 2—Electron micrograph of yolk with granules.

Solubility index of protein

200 mL of granular protein suspension was centrifuged for 20 min at 4,000 × *g*, and the protein concentration of the resulting supernatant fluid was determined by the Kjeldahl method. The solubility index of protein was expressed as:

$$SI\ (\%) = \frac{\text{Protein concentration of supernatant fluid (mg/L)}}{\text{Initial protein concentration (mg/L)}} \times 100$$

Viscosity

The granular suspensions had a Newtonian behavior (at the concentrations we used) so the viscosity was measured by a capillary tube viscometer (Viscoboy, Lauda-Königshofen, Germany).

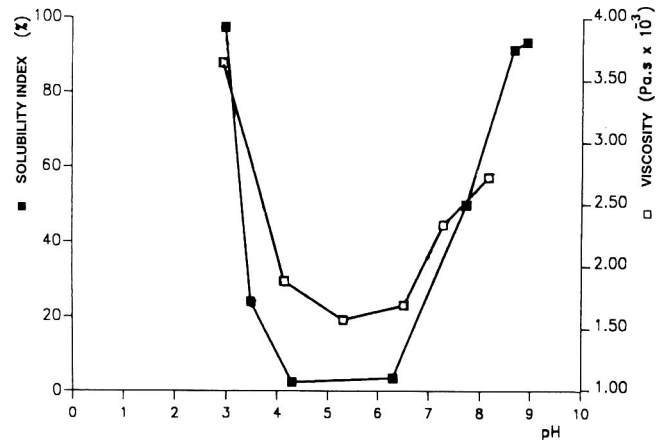


Fig. 3—Effect of pH on solubility and viscosity of granular proteins (protein concentration = 2%).

Table 2—Solubility index of granular proteins after modification of pH or ionic strength (protein concentration = 2%)

	Solubility index
pH = 6.4	3.4
pH = 3	100.0
pH = 9	100.0
pH = 3 to pH = 6.4	4.0
pH = 9 to pH = 6.4	3.7
+ 0.58M NaCl	98.9
+ 0.58M NaCl then dialyzed (24 hr)	16.6

RESULTS & DISCUSSION

Composition and ultrastructure of granules

Granules represented about 25% of the dry weight and nearly 50% of the protein content of the yolk (Table 1). The lipid content was limited, since the ratio of lipid/protein was 0.56 compared to 1.96 in yolk. The differences observed, in comparison to the results obtained by Burley and Cook (1961), could be explained by the different methods used to isolate the granules.

The polyacrylamide gel electrophoregrams (Fig. 1) separated the characteristic bands of the three proteins. Their ratios were determined by Burley and Cook (1961) using ultracentrifugation. Although we found no published results for electrophoresis relative to granules, work by Chang et al. (1977), McBee and Cotterill (1979), Dixon and Cotterill (1981) and Woodward and Cotterill (1987) enabled us to identify the main bands. In our polyacrylamide gel electrophoretic patterns, we determined 11 bands. The first represented low-density lipoprotein (LDL). On account of their high molar mass and their low charge, those proteins did not pass through the polyacrylamide gel. Moreover, the high lipid content would account for the low staining of the bands. Bands 2 and 3 corresponded to lipovitellin. Bernardi and Cook (1960) isolated lipovitellin in α and β fractions which differed by their phosphorus content (0.48% and 0.27%, respectively) but had the same molar mass. Lipovitellin α (band 3) had more phosphorus content, and, therefore, it migrated faster than the β fraction (band 2). Lipovitellin α was predominant in the granules. Bands 10 and 11 were characteristic of α- and β-phosvitin. Band 10 corresponded to β-phosvitin and band 11 to α-phosvitin. Their phosphorus contents were α 9.2% and β 2.97% (Abe et al., 1982). However, α-phosvitin migrated faster than β-phosvitin, because its molar mass was lower (Clark, 1970; Abe et al., 1982).

Divalent and trivalent cations were highly concentrated in the granules as shown by atomic absorption spectrophotometry (Table 1); 81% of the calcium, 71% of the magnesium and 98% of the iron were found in the granules. Several researchers

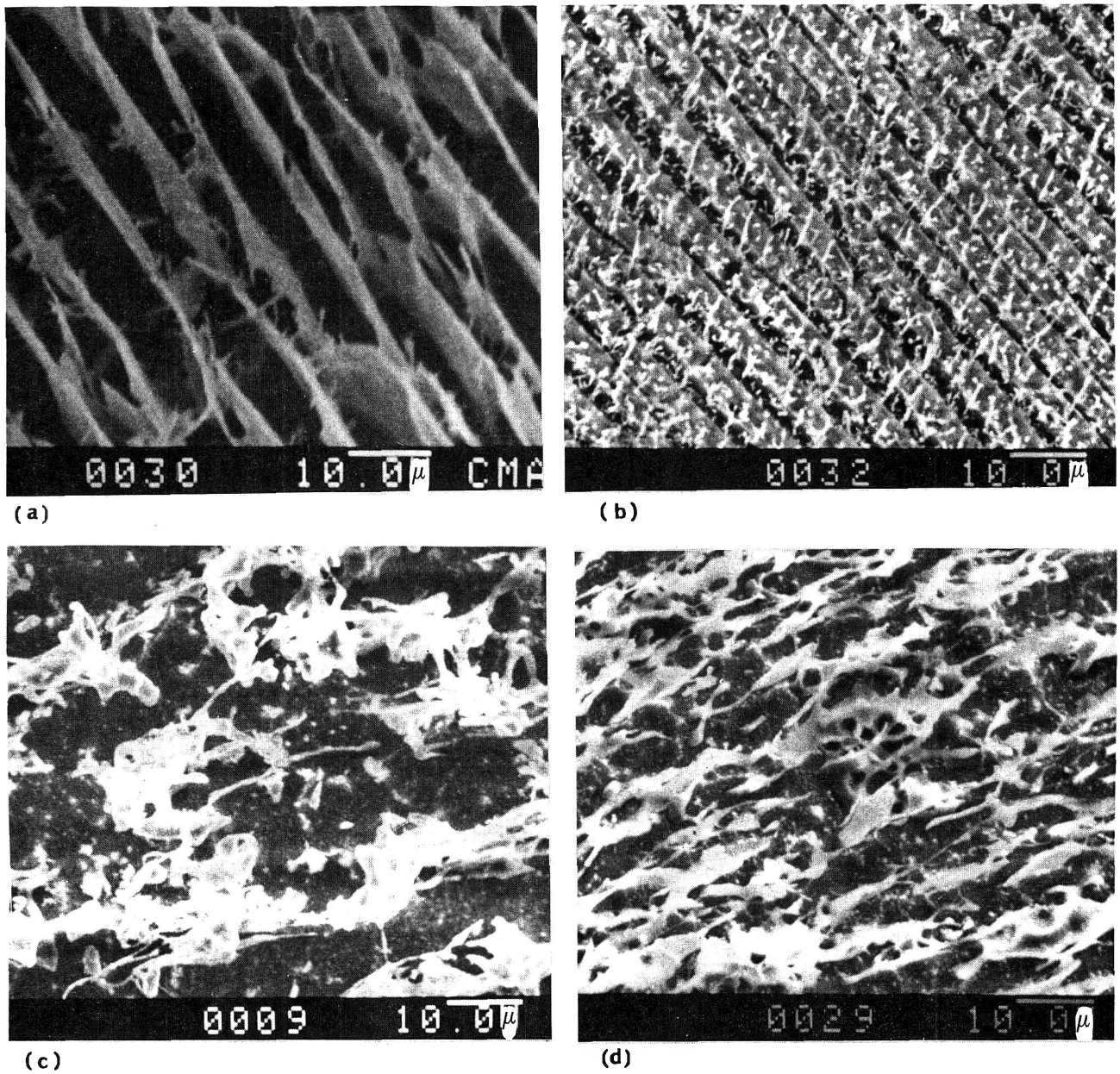


Fig. 4—Electron micrograph of granular fractions at (a) pH = 3, at (b) pH = 9, at (c) pH = 3 to 6.4 and at (d) pH = 9 to 6.4.

have shown the important binding capacity of mineral elements by phosvitin. This is due to the existence of many serine-phosphate residues in this protein (Mecham and Olcott, 1949; Grizzuti and Perlmann, 1973; Webb et al., 1973; Weller, 1979).

These cations might play a role by binding charged groups as they do in casein micelles. They might constitute ionic bridges between the phosphate groups of the phosvitin, lipovitellin and LDL. Indeed, phosvitin contains 10% phosphorus, completely bound to protein (Joubert and Cook, 1958). The α-lipovitellins contain 0.48% and β- 0.27% phosphorus. (Bernardi and Cook, 1960), associated with the lipid fraction and with the seryl residues (Belitz, 1963). The phosphorus atoms of LDL are located on the phospholipids at the surface of the micelles (Osuga and Feeney, 1977).

Scanning electron microscopy was used to visualize the ultrastructure of egg yolk, particularly the granule units. Figure 2 shows the shape of granules. Size ranged from 0.2 to 2 μm. A similar value was reported by Bellairs (1961) using electron microscopy and by Chang et al. (1977) using a Coulter counter.

Effect of pH on structure of granules

At the initial pH of the mixture (pH = 6.3), lipovitellins, phosvitins and LDL were associated with insoluble granules. Acidification and alkalization caused the dissociation and complete solubilization of these proteins. At acidic pH (pH <4.2), the decrease of phosphate monoester residues, the ionization of the carboxylic groups and the increase of repulsive forces between the positive charges (NH_3^+) is believed to induce the break-up of ionic bridges. At basic pH (pH > 6.3), the increase in number of negative charges (COO^-) induced electrostatic repulsions presumably resulting in destruction of the granules. The change of viscosity with pH (Fig. 3), caused by the change of solubility, is explained by electrostatic repulsion existing at extremes of pH. When the granules were dissociated at an acidic or basic pH and then brought back to the initial pH, they recovered their initial low solubility (Table 2). This was not due to an isoionic precipitation of proteins, because, in the case of basic pH, the insolubility occurred without passing through the isoelectric pH of the different proteins. Neverthe-

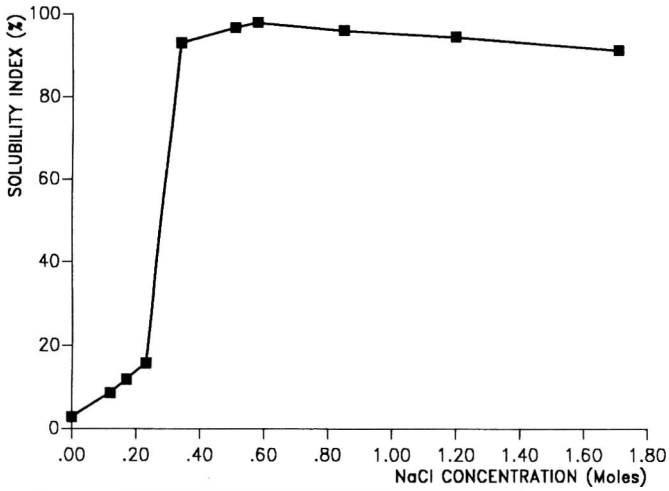


Fig. 5—Effect of NaCl concentration on the solubility of granular proteins (protein concentration = 2%).

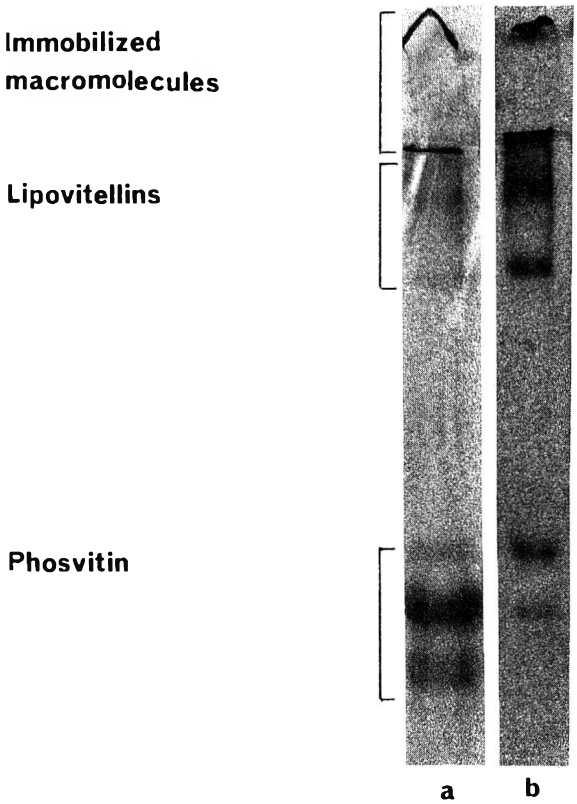
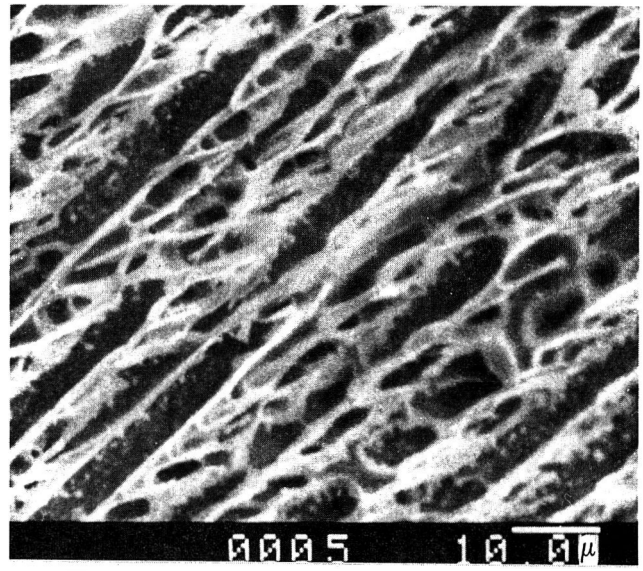
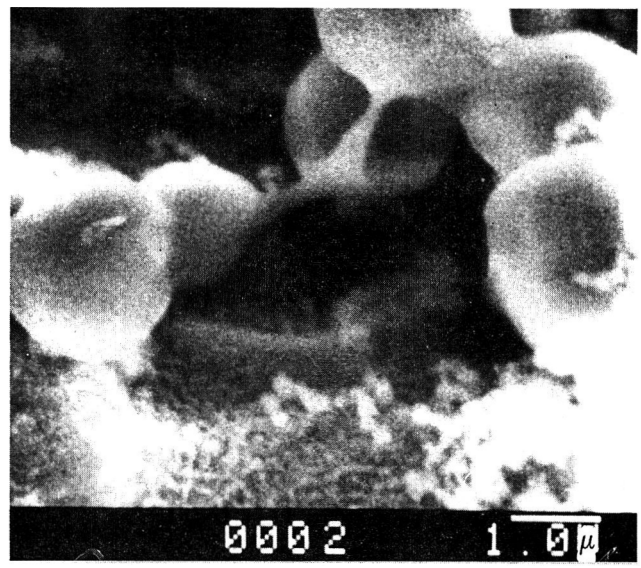


Fig. 6—Polyacrylamide-gel electrophoretic pattern of (a) soluble and (b) largely insoluble proteins of the granular fraction which was salted and dialyzed.



(a)



(b)

Fig. 7—Electron micrograph of granular fraction (a) in 0.58M NaCl and (b) in a dialyzed dispersion.

less, phosphocalcic bridges could exist and cause the loss of solubility of reassociated constituents.

Observations by scanning electron microscopy (Fig. 4a, b, c, d) made after the modification of pH did not show the granular structure seen at pH = 6.3. At acidic (Fig. 4a) and basic (Fig. 4b) pH, we observed a network which might be due to freezing. In fact, the lack of microstructure in these solutions facilitated the growth of ice crystals and the formation of the network. The different sizes of the mesh at pH = 3 and pH = 9 were explained by the different charges of the proteins and by passing the isoelectric point (pH_i) at pH = 3. After the change of pH, the network disappeared. The proteins were randomly associated. The granules did not restructure themselves; however, the bonds which existed between the proteins could be the same as those responsible for the granular unit.

Effect of ionic strength on integrity of granular structure

At the initial pH (pH = 6.4), the granules were progressively dissociated when the ionic strength was increased by addition of sodium chloride. Chang et al. (1977) observed the total dissociation of granules when adding 1.71M NaCl to the yolk. According to our results, whatever the concentration of protein, this dissociation occurred when the concentration of NaCl reached 0.58M (Fig. 5). This observance could be attributed to the substitution of monovalent sodium for bivalent cations. This substitution could lead to a break-up of the phosphocalcic bridges and subsequent calcium release. The affinity constant of sodium towards phosphates was lower than that of calcium, magnesium and iron for any studied protein (Dickinson and

Perkins, 1971; Grizzuti and Perlmann, 1973). The addition of a large excess of sodium would allow the movement of bivalent cations. In Fig. 5, 52 moles of sodium/mole of calcium were added to break the granules from a solution of 2% protein.

However, when a solution of granules destructured by NaCl was dialyzed against water, the solubility index of the proteins decreased (Table 2). The polyacrylamide gel electrophoregrams (Fig. 6) showed that phosvitin was the only soluble protein. With the dialysis, the elimination of the weakly linked sodium brings LDL and lipovitellins to their pH_i where they are precipitated by electrostatic attractions. Changes in the solubility of phosvitin were caused by the large number of negative charges associated with the phosphoseryl residues, which constituted nearly a third of the residues of the protein (Allerton and Perlmann, 1965). According to Burley and Cook (1961), phosvitin represented 17% of the proteins in the granule. This percentage corresponded exactly to that of soluble proteins after salting and dialysis.

The scanning electron micrograph (Fig. 7a) shows the effect of the increase of ionic strength on the microstructure of granules. For acidic or basic pH, the destruction of granules led to the formation of an orientated network during freezing. When granules were dissociated by NaCl and then dialyzed, spherical units could be observed on the micrograph (Fig. 7b). These could be formed by the association of LDL and lipovitellins. The size of these "pseudo-granules" was about 2 μm, larger than the diameter of ordinary granules. The increase of the diameter could be due to the decrease of the number of phosphocalcic bridges due to the loss of phosvitin.

CONCLUSIONS

THE MICROSTRUCTURE of egg yolk granules closely depends on pH, ionic strength and presence of bi- or polyvalent mineral cations. The modification of one of these could lead to an irreversible destruction of the granules. The high concentration of bivalent cations in yolk and the presence of phosphoproteins (mainly phosvitin) caused us to hypothesize the existence of ionic bridges between the cations and the phosphate groups of the phosphoseryl residues of phosphoproteins. These would operate in the same way as those present in the casein micelle. The change in behavior of the granules when pH or ionic strength were modified supported the existence of this type of bond. Further studies on the role of bi- or trivalent cations would allow a better understanding of the granule microstructure and of its effects on functional properties of yolk.

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Fractionation of Water-Soluble and -Insoluble Components from Egg Yolk with Minimum Use of Organic Solvents

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ABSTRACT

A process was developed to fractionate both water-soluble and water-insoluble components from egg yolk with maximum retention of biological and functional properties. About 60–90% of the immunological activity of yolk was recovered in a supernatant after centrifugation of yolk diluted six- to tenfold with water. The remaining yolk pellet had excellent emulsifying properties for mayonnaise preparation. It was further fractionated by deoxycholate treatment to separate triglycerides (80% recovery). Ultrafiltration of the deoxycholate supernatant yielded protein (74% recovery) in the retentate, and phospholipids and cholesterol in the permeate. Phospholipids were then purified by dichloromethane:ethanol (3:1) extraction, zinc chloride precipitation and acetone washing.

Key Words: fractination, egg yolk, lipids, proteins

INTRODUCTION

PROTEINS AND LIPIDS are the major constituents of egg yolk. Both fractions play important roles in the food processing industry as well as in cosmetic formulations and as bioactive compounds in pharmaceutical products (Szukaj, 1983). The most plentiful constituent in egg yolk solids is the lipid fraction, which constitutes roughly one-third of the yolk. Triglycerides (TG) are the predominant neutral lipids, followed by various phospholipids (PL) consisting mainly of phosphatidyl choline or lecithin, then by cholesterol, a relatively minor constituent in egg yolk. Proteins constitute 15–17% of the yolk, including phosvitin, α- and β-lipovitellins and low-density lipoproteins in the yolk granule, and α-, β- and γ-livetins as well as lipoproteins in the yolk plasma (Powrie, 1976). γ-Livetin has been recognized as an IgG-like antibody (Polson et al., 1980), and is also commonly referred to as yolk immunoglobulin or IgY.

Due to the ready availability of egg yolk as a good source of lecithin, IgY, as well as other bioactive or functional compounds, many attempts have been made to isolate these components. However, it is difficult to recover the lipid components without denaturing or inactivating the protein components. The conventional process for lecithin and lipid extraction utilizes organic solvents, whereby proteins are inevitably denatured (Kolarovic and Fournier, 1986). An alternative to conventional solvent extraction procedures for separating lecithin and cholesterol from egg oil is the use of super critical fluid extraction (SCFE). However, even under those conditions, some proteins were denatured (Weder, 1984). Since egg yolk is a mixture of lipids, lipoproteins and water-soluble proteins, the water-soluble fraction including IgY could be extracted in undenatured form with water before further fractionation of the water-insoluble components. Some attempts have been made to separate γ-livetins from egg yolk using polyethylene glycol (Polson et al., 1980, 1985) and sodium or dextran sulfate (Jensenius et al., 1981)

The objective of our study was to establish a process for recovery of biologically active or functional components from both water-soluble and -insoluble fractions of egg yolk while avoiding exposure of protein components to organic solvents. A specific objective was to develop a mild process for separation of a water-soluble fraction containing immunologically active IgY. The remaining water-insoluble yolk fraction could still be used in food applications which require its emulsifying functionality (for example, as an ingredient in mayonnaise formulations). Further fractionation of the water-insoluble yolk fraction to triglycerides, protein, phospholipid and cholesterol components was also investigated.

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MATERIALS & METHODS

Materials

Hen eggs (3 batches) were obtained from the University of British Columbia animal farms. The freshly laid eggs were either used within about a wk. after collection (termed "fresh" eggs) or after 6–8 wk at 4°C ("stored" eggs). One batch of eggs was also purchased from a local store. Anti-chicken IgG antisera, chicken IgG and sodium deoxycholate (NaDOC) were purchased from Sigma Chemical Co. (St. Louis, MO). Sodium alginate (Kelgin XL) was obtained from Kelco Co. (Toronto, Ontario). Immunodiffusion plates were purchased from ICN (Cleveland, OH), Kieselgel Thin Layer Chromatography (TLC) and High Performance (HP) TLC plates were purchased from Terochem Laboratories Ltd. (Edmonton, Alberta). Ultrafiltration membranes (XM50, molecular weight cutoff 50,000) were from Amicon Corporation (Danvers, MA).

Methods

Fractionation of egg yolk. The general scheme for step-wise separation of IgY, TG, proteins, cholesterol and lecithin from egg yolk is shown in Fig. 1. Three or more trials were performed for each step in the process, using either "fresh" eggs or "stored" eggs. Details of each step are described in the Results section, with the final recommended process as follows.

Separation of water-soluble proteins from egg yolk—Egg yolk was diluted 10X with distilled water and held overnight at 4°C before centrifuging at 10,000 × *g* for 15 min to separate water-soluble proteins in the supernatant from the remaining yolk pellet.

Separation of egg yolk triglycerides from yolk pellet—NaDOC (0.92 gm/mL egg yolk) was added to the egg yolk pellet obtained in Step 1, which had been resuspended with the aid of manual stirring in 0.0625 M sodium carbonate buffer, pH 8.2, containing 0.0625M sodium chloride. The mixture was held for about 4.5 hr at room temperature and then centrifuged at 10,000 × *g* for 15 min to separate a creamy top layer (TG) from the bottom amber supernatant (AS).

Separation of proteins from AS—AS, two times diluted with distilled water, was ultrafiltered through an Amicon XM50 membrane. The retentate containing the protein fraction was washed once with 0.01 M NaDOC, ultrafiltration continued, and the permeates were pooled.

Purification of PL from permeate fraction—Crude PL was separated from NaDOC in the permeate from Step 3 by extracting with ethanol:dichloromethane (EtOH:CH_2Cl_2 in a 1:3 volume ratio). The CH_2Cl_2 layer, containing the PL, was evaporated using a rotary evaporator at 25°C followed by final evaporation under a stream of nitrogen. EtOH (95%) was added to redissolve the dried crude PL before purifying essentially according to the method of Hatta et al. (1988), with addition of $ZnCl_2$ to a concentration of 1–2% (w/v). The mixture was held at 4–5°C for 60 min and then centrifuged at 3000 × *g* for 10 min. The supernatant was found to contain most of the cholesterol,

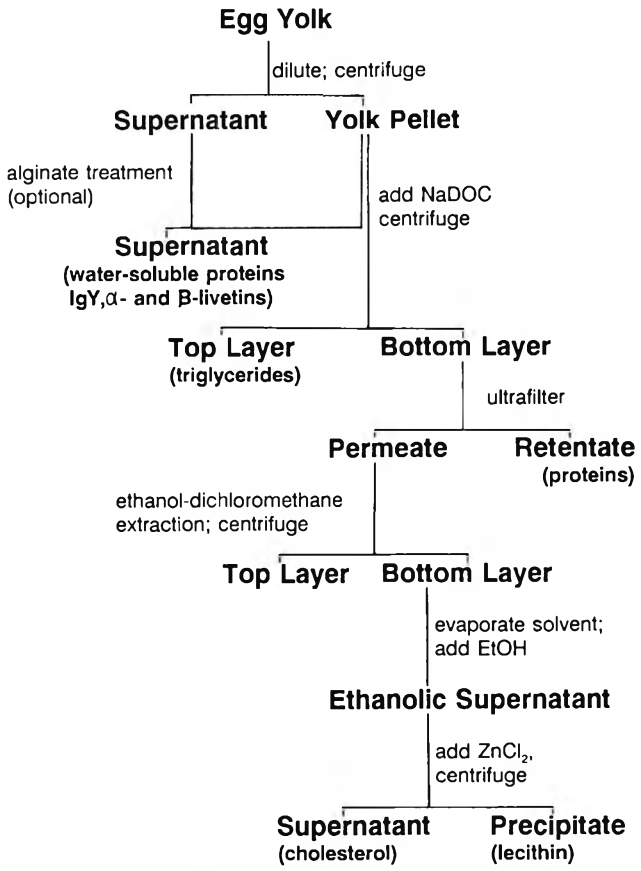

Fig. 1—Flow chart for separation of IgY, proteins, triglycerides and lecithin from egg yolk.

while the precipitate (mostly PL) was further purified by washing with pre-cooled acetone.

Centroid mapping optimization. Centroid mapping optimization design (Aishima and Nakai, 1986) was used to develop conditions for Step 2 in the fractionation process to achieve best separation of yolk triglycerides from the water-insoluble yolk pellet fraction. The objective was to separate TG as a floating creamy layer, that is, to minimize amount of TG remaining in the bottom amber supernatant (AS) after centrifugation of the NaDOC-treated mixture. The response value R used for minimization was a ratio of TG in the amber supernatant to TG in the pellet, where TG was calculated as the difference between total lipid and phospholipid content in each fraction. The experimental conditions under investigation were dilution of the yolk pellet, concentration of NaDOC, and incubation pH, temperature and time. The conditions at each vertex in the optimization are described under Results.

Radial immunodiffusion. Immunodiffusion plates were prepared as described by Williams and Chase (1971) with some modification. Anti-chicken IgG antiserum (0.35 mL) was mixed with 1.65 mL phosphate buffer saline (PBS) at pH 7.0 before mixing with 5.0 mL 1% agarose containing 0.02% sodium azide in PBS. The plate was allowed to solidify by standing for 5 min at room temperature before transferring to a moist chamber for storage at 4°C until used. Wells (3 mm diameter) were cut using a template. Three uL samples and standards with concentration in the range 0.5 to 2.0 mg IgG/mL were applied to the wells. Diffusion in a moist chamber at room temperature was considered complete when the ring diameter did not further increase. Quantification of IgG in samples was based on the diameter of the precipitin ring compared to those measured for IgG standard solutions of known concentration.

Mayonnaise preparation. Laboratory-prepared mayonnaise was made by a modified method of Yang and Cotterill (1989). The original formulation contained 80 g egg yolk, 15g salt, 10.1g mustard, 144.6g vinegar and 747g oil. For mayonnaise made with resuspended egg yolk pellet obtained after removal of the water-soluble fraction, the formulation was modified to provide an equal amount of total yolk solids and to maintain correct pH. The yolk pellet was resuspended in water to the original yolk volume, using homogenization at 5000 rpm, for 30 sec at room temperature (Ultra-Turrax Homogenizer, Janke and Kunkel, Edmonton, Alberta). Instead of using 80g untreated egg yolk and 144.6g vinegar in the formulation, 113.5g resuspended yolk pellet and 1.68g acetic acid plus 109.42g vinegar were used. The emulsion was formed using a KitchenAide Model k5-A mixer (Hobart Co., Troy, OH). The consistency of the prepared mayonnaise was determined using the Bostwich consistometer, by measuring the distance flowed in a given period of time.

Table 1—Effect of dilution and pH on the separation of lipids from water-soluble fraction of egg yolk

Dilution factor	Ilpid in supernatant (%)[a]		Appearance of supernatant[b]	
	pH 6.0	pH 7.0	pH 6.0	pH 7.0
2	97	93	O, Y	O, Y
4	79	86	O, Y	O, Y
6	31	55	O, sl.Y	O, Y
8	10	38	W, T	T, sl.Y
10	7	31	W, sl.T	T, sl.Y

[a] based on 29% lipid content in control (not centrifuged) egg yolk.
[b] O = opaque, Y = yellow, W = white and T = turbid.

Chemical analysis. Protein content was determined by a modified Lowry method according to Markwell et al. (1978). Lipids were extracted in chloroform:methanol (3:1) and total lipids determined gravimetrically after evaporation of solvent. PL in the extract were determined according to the method of Raheja et al. (1973); deoxycholate was extracted according to Helenius and Simons (1972) and quantified according to Szalkowski and Mader (1952). Cholesterol content was measured fluorometrically (Roberts, 1968). All analyses were performed at least in duplicate.

Emulsification activity index (EAI). EAI (m^2/gram solids) was determined based on the methods of Pearce and Kinsella (1978) and Mizutani and Nakamura (1985).

Thin-layer chromatography (TLC). Qualitative analysis of lipid extracts was carried out by TLC on Kieselgel 60 pre-coated and High-Performance Kieselgel 60 pre-coated plates. The plates were successively developed with chloroform-methanol-water (65:25:4) and hexane-ether (4:1). Separated components were made visible by spraying with 55% (w/v) sulfuric acid containing 0.6% $K_2Cr_2O_7$ and then charring at 120°C for 20 min.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). SDS-PAGE was performed using a Mini-Protean II Dual Slab Cell (Bio-Rad Laboratories, Mississauga, Ontario). A 7.5% acrylamide separating gel and a 3% acrylamide stacking gel were prepared according to Laemmli (1970). Staining with Coomassie Brilliant Blue R-250 and destaining procedures were according to Bio-Rad's instruction manual section 12.5.

RESULTS & DISCUSSION

Separation of water-soluble fractions, including IgY, from egg yolk

Table 1 shows the effect of two- to tenfold dilution of egg yolk with water at either pH 6.0 (unadjusted) or 7.0 (adjusted with 1.0 N NaOH) on the percent lipids found in the water-soluble fraction of egg yolk. The objective was to retain most of the lipids in the egg yolk pellet while recovering immunoglobulin (IgY) in the water-soluble fraction (supernatant) after centrifugation. Hence the best condition (Table 1) for separation of lipids into the pellet was at pH 6.0, dilution ratio 1:9, or ten times dilution. Under these conditions, over 90% of the total lipids and PL in the original egg yolk were recovered in the pellet, using eggs which had been stored for at least 6 wks. after laying. The recovery of lipids in the pellet declined to 70% when eggs obtained shortly after laying were used in the process. Reasons for the more efficient separation of lipids after storage of the eggs are not known. Possibly the loss of water through evaporation or loss of sulfhydryl (SH) groups which can occur during cold storage (Burley and Vadehra, 1989) may affect separation of water-soluble and -insoluble fractions. Lipoproteins (LP), which make up a large proportion of the egg yolk, are inherently unstable in the absence of water and contain a small proportion of sulfhydryl groups (Burley and Vadehra, 1989). Thus the changes in water content and/

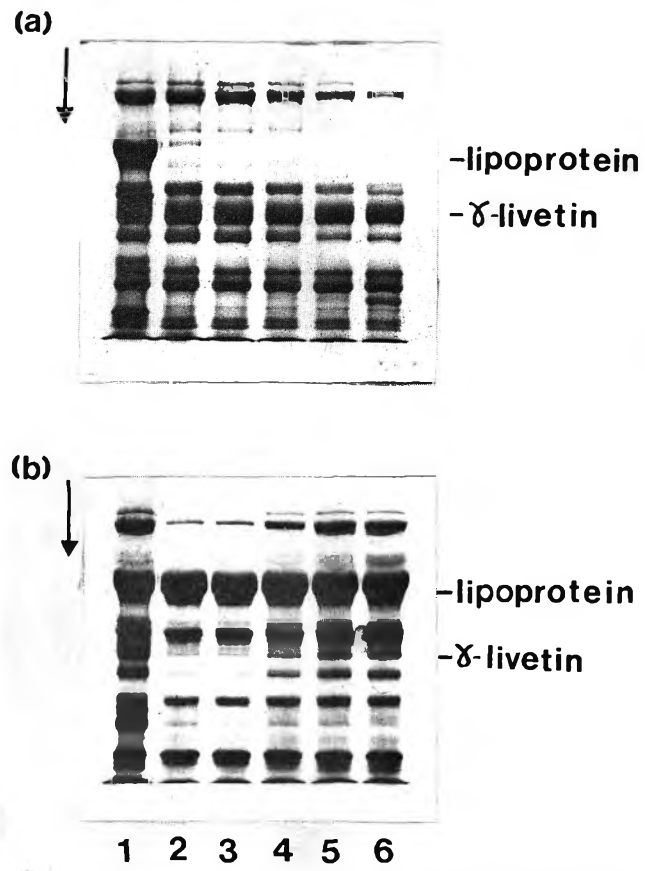

Fig. 2—SDS-PAGE profiles of whole egg yolk and (a) the supernatant and (b) the pellet fractions of diluted yolk (pH 6.0) after centrifugation. (a) lane 1=control egg yolk; lanes 2–6=supernatants from 2, 4, 6, 8 and 10X dilutions, respectively; (b) lane 1 = control egg yolk; lanes 2–6=pellets from 2, 4, 6, 8 and 10X dilutions, respectively. The final dilution factor of samples for application to SDS-PAGE was 20 times.

or SH groups occurring during cold storage could affect separation of yolk lipids. Nevertheless, the lipids (30%) remaining in the water-soluble fraction after centrifugation of diluted yolk from fresh eggs can be separated from the protein fraction by using sodium alginate, according to Hatta et al. (1988). At a concentration of 0.05% sodium alginate, 98% of the PL found in the supernatant was precipitated and could then be added back into the egg yolk pellet (water-insoluble fraction) for use as a food ingredient or for further fractionation.

Figures 2a and b show the SDS-PAGE profiles of whole egg yolk (diluted but not centrifuged) and the supernatant (2a) and pellet fractions (2b), from two- to tenfold diluted egg yolk after centrifugation. The majority of the lipoproteins, the major non-aqueous constituent of egg yolk granules, was found in the pellet fractions (Fig. 2b, lanes 2–6). γ-Livetin or IgY and other livetins tended to be in the water-soluble supernatant fractions (Fig. 2a, lanes 2–6). However, IgY appeared in trace quantities in the pellet from sixfold diluted yolk and in slightly higher quantities in the pellets from eight- and tenfold diluted yolk (Fig. 2b, lanes 4–6). Water-soluble proteins constitute 42.4% of the total proteins in egg yolk (Osuga and Feeney, 1977). Under the tenfold dilution condition, about 15–21% of the total protein was recovered in the supernatant, including about 60% recovery of IgY activity. The amount of protein recovered in the supernatant decreased with increasing dilution. Recovery of IgY decreased from about 100 to 60% in the supernatants from six- and tenfold diluted yolk, respectively. However, the purity of IgY recovered in the supernatant was higher with increasing dilution factor, probably due to greater removal of other protein components into the pellet fraction (Table 2).

Table 2—IgY activity of the supernatants from 6× and 10× diluted EY (pH 6.0)

	Ig recovery[a] (%)	Protein recovery[b] (%)	Ig purity[c] (g Ig/100g protein)
EY control	100	100	6
6× sup.	100	48	14
10× sup.	60	21	18

[a] IgY activity of diluted supernatants (sup.) as a percentage of that of control egg yolk (7.6 mg IgY/mL), determined by radial immunodiffusion.
[b] Protein content of diluted sup. as a percentage of that of control egg yolk (12.5%), determined by the modified biuret-phenol method.
[c] Purity calculated as the percent ratio of active IgY to total protein in each sample.

Table 3—Optimization of conditions for separation of triglycerides (TGs) from egg yolk (EY) with sodium deoxycholate (NaDOC)

	Ranges of each factor					
	Initial simplex		First simplex-centroid		Second simplex-centroid	
Factors	LL[a]	UL	LL	UL	LL	UL
pH	8.0	12.0	8.0	9.5	8.0	9.0
Temperature (T°C)	20.0	50.0	20.0	30.0	23.0	30.0
Dilution factor	5.0	20.0	8.0	10.0	7.0	9.0
NaDOC (g/mL EY)	0.5	2.5	0.9	1.0	0.9	1.0
Time (hr)	0.5	5.0	4.0	6.0	4.0	6.0

	Experimental conditions					
Vertex #	pH	T°C	Dil.	NaDOC	Time	Response[b]
(Initial simplex)						
1	8.00	20.00	5.00	0.50	0.50	100.00
2	11.65	26.15	8.08	0.91	1.42	42.35
3	8.82	47.36	8.08	0.91	1.42	100.00
4	8.82	26.15	18.68	0.91	1.42	100.00
5	8.82	26.15	8.08	2.32	1.42	100.00
6	8.82	26.15	8.08	0.91	4.60	28.58
(First s mplex-centroid)						
1	8.00	20.00	8.00	0.90	4.00	19.96
2	9.37	22.05	8.41	0.92	4.41	22.27
3	8.31	29.12	8.41	0.92	4.41	16.20
4	8.31	22.05	9.82	0.92	4.41	26.03
5	8.31	22.05	8.41	0.99	4.41	15.47
6	8.31	22.05	8.41	0.92	5.82	18.61
7	8.46	23.05	8.33	0.93	4.61	20.58
8	8.61	24.06	6.83	0.94	4.81	17.25
9	8.77	25.13	5.23	0.95	5.03	100.00
(Second simplex-centroid)						
1	8.00	23.00	7.00	0.90	4.00	19.39
2	8.90	24.44	7.41	0.92	4.41	14.43
3	8.20	29.39	7.41	0.92	4.41	14.56
4	8.20	24.44	8.82	0.92	4.41	12.55
5	8.20	24.44	7.41	0.99	4.41	14.89
6	8.20	24.44	7.41	0.92	5.82	18.03
7	8.35	25.43	7.69	0.94	4.69	18.38
8	8.34	25.62	7.75	0.94	4.47	18.08
9	8.41	25.86	7.82	0.93	4.48	17.51
10	8.45	25.16	7.90	0.93	4.49	18.08
11	8.36	25.30	8.00	0.93	4.51	18.36
12	8.39	25.47	7.83	0.93	4.53	18.08

[a] LL=lower limits; UL=upper limits
[b] Response = [total lipids (mg) in AS - PL (mg) in AS]/[total lipids (mg) in EY pellet - PL (mg) in EY pellet].

Based on these results, eight- to tenfold dilution would be preferred for more efficient separation of lipid (90–93%) from the water-soluble fraction, with moderate (60%) recovery of IgY. For more complete recovery of IgY, but with less complete separation from lipids, sixfold dilution may be used.

Separation of yolk triglycerides (TG) from water-insoluble pellet

Table 3 shows the series of optimization experiments designed to develop conditions yielding maximum separation of TG from the egg yolk pellet using NaDOC. In this optimization, the search was first conducted with an initial simplex

Table 4—Phospholipids and sodium deoxycholate contents in the permeate, ethanol and dichloromethane fractions from EtOH:CH_2Cl_2 (1:3) extraction

Fractions[a]	Sample vol. (mL)	NaDOC (g)	Phospholipids (g)
P	80	3.28	0.158
EtOH-H_2O	100	3.00	0.009
CH_2Cl_2	230	0.028	0.147

[a] P = permeate; EtOH-H_2O = ethanol phase; CH_2Cl_2 = dichloromethane phase

Table 5—Efficiency of $ZnCl_2$ precipitation and acetone washing on phospholipid (PL) purification

Fractions[a]	PL (mg)	Recovery of PL (%)	Descriptions[b]
Crude PL	147	100	Cl, Y
S_1	27	18	Cl, Y
S_2	33	22	Cl, sl. Y
S_3	85	58	Cl, C

[a] Crude PL = dichloromethane (CH_2Cl_2) layer; S_1 = supernatant from $ZnCl_2$ precipitation; S_2 = acetone wash; S_3 = purified PL.
[b] Cl = clear; Y = yellow; C = colorless.

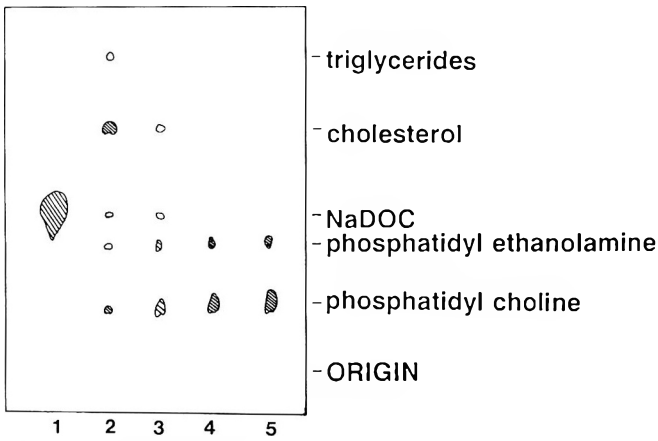

Fig. 3—HPTLC of fractions from CH_2Cl_2:EtOH extraction, supernatant from $ZnCl_2$ precipitation (S_1), acetone wash (S_2) and purified PL (S_3). Lane 1 = EtOH—H_2O layer from CH_2Cl_2:EtOH extraction of the permeate; lane 2 = $ZnCl_2$ supernatant(S_1); lane 3 = acetone wash (S_2) and lanes 4–5 = purified PL (S_3) 10 μL and 20 μL, respectively. Density of the slant line represents the relative intensity of spots visualized.

constructed with broad ranges for the factor levels, then followed by two simplex-centroid searches. The broader search area used in the first initial simplex allowed us to determine an appropriate narrower area for finer search of the optimum conditions in the subsequent simplex-centroid searches.

The main search areas for the first initial simplex were defined by the following lower and upper limits of each factor: pH 8–12, temperature 20–50°C, dilution of the yolk pellet 5–20 times, concentration of NaDOC 0.5–2.5 g/mL egg yolk and incubation time 0.5–5.0 hr. Six vertices were generated from the search. The experimental conditions generated for vertex 6 gave the lowest value, i.e. best response (28.58). Based on this response, narrower ranges or boundaries for the five factors were estimated and experimental conditions for the initial simplex and centroid search were calculated as shown in Table 3. Best conditions found from the first simplex-centroid search were those of vertex 5, giving a minimum response of 15.47. A second simplex-centroid search was conducted to determine if even better separation of TG could be achieved by searching for the optimum or minimum response value within even closer boundaries. A small improvement was achieved. Vertex 4 of the second simplex-centroid search had a response value of 12.55, a slight improvement from 15.5 found in the first simplex-centroid search.

Table 6—Emulsifying activity index (EAI) of egg yolk control (EY) and yolk pellet remaining after removal of water-soluble fraction from tenfold diluted yolk (P)

Emulsification conditions	EAI, m^2/g solids[a]	
	EY	P
3% total solids		
0% NaCl	8.1[b]	9.5[c]
2% NaCl	8.5[b]	9.8[c]
6% total solids		
0% NaCl	5.0[b]	5.8[c]
2% NaCl	4.8[b]	5.9[c]

[a] Average values of two replicates.
[b,c] Numbers in the same row bearing different superscripts are significantly different ($P < 0.01$).

Thus the best result, achieved after 27 experiments, was a response value of 12.55, representing about 80% TG separation into the upper layer and 80% of the yolk proteins and 84% of the PL, remaining in the amber supernatant. The experimental conditions yielding this result were pH 8.2, 24.4°C, dilution factor of egg yolk pellet 8.8 times, NaDOC 0.92 g/mL egg yolk and incubation 4.4 hr.

Separation of yolk proteins from amber supernatant (AS)

Twice diluted AS was ultrafiltered through an Amicon XM50 membrane (MW cutoff 50,000). About 74% of the proteins was retained in the retentate, while most of the PL (78%), NaDOC (78%) and cholesterol (68%) were recovered in the permeate.

Purification of phospholipids (PL) from the permeate

Direct purification of PL from the permeate was initially attempted using zinc chloride precipitation according to Hatta et al. (1988). However, preliminary trials indicated that, at concentrations of 2% or higher, the NaDOC used in Step 2 for separation of TG from the yolk pellet interfered with $ZnCl_2$ precipitation of PL. A solvent extraction procedure was therefore investigated to separate NaDOC from the lipid fractions. Several solvents were investigated, including acetone, hexane, 5% ethyl acetate in hexane, hexane:isopropanol (2:1), ethyl acetate:isopropanol (4:1), chloroform:methanol (3:1) and ethanol:dichloromethane (1:3). The best combination involved the food use-approved solvents ethanol (EtOH) and dichloromethane (CH_2Cl_2) at a volume ratio of 1:3 per volume of permeate to be extracted. Under those extraction conditions, more than 90% of the PL was extracted into the CH_2Cl_2 layer leaving almost all of the NaDOC in the alcohol layer (Table 4). After evaporating the CH_2Cl_2 and redissolving the crude PL in 95% EtOH, further purification of PL could then be achieved by $ZnCl_2$ precipitation as previously reported by Hatta et al. (1988) and Von Glos and Boursnell (1981). Table 5 shows the efficiency of $ZnCl_2$ precipitation for purification of the crude PL obtained from CH_2Cl_2-EtOH extraction. The $ZnCl_2$ supernatant contained 65% of the yolk cholesterol. The precipitate was washed with pre-cooled acetone, and found to contain 40–45% of the yolk PL which was of high purity based on TLC analysis (Fig. 3, lanes 3–4).

Emulsifying property of egg yolk after removal of water-soluble fraction

The emulsifying components in yolk are PL, lipoproteins and proteins. The emulsifying function of egg yolk pellet remaining after removal of the water-soluble fraction for IgY recovery was determined by EAI in a model system, as well as by its ability to be used as an ingredient for mayonnaise preparation.

Using the model emulsion system of yolk solids and corn oil, the EAI of the treated egg yolk was slightly higher ($P < 0.01$)

than that of the control, untreated egg yolk, in the absence and presence of 2% salt (Table 6). The high EAI value of the treated egg yolk suggested that it would be a useful functional ingredient in food application. This was demonstrated in the preparation of mayonnaise. Due to removal of the water-soluble constituents, yolk pellet resuspended to the original yolk volume had a lower total solids content than the untreated yolk (0.38 vs 0.54 grams/mL, respectively). Mayonnaise prepared using resuspended yolk pellet according to the original mayonnaise formulation was too thin for consumer acceptance. However, the formulation was modified to compare use of the yolk samples on an equivalent solids basis. The consistency of the mayonnaise prepared from resuspended egg yolk pellet was thicker than that of the control mayonnaise prepared from untreated egg yolk. According to the consistometer measurements, mayonnaise prepared from resuspended yolk pellet flowed 0.5 cm in 30 sec, compared to 5.3 cm for mayonnaise prepared from control untreated yolk. These results demonstrated the superior emulsifying functionality of the yolk pellet remaining after removal of the water-soluble fraction. Due to the higher EAI of the yolk pellet, as shown in Table 6, further modification of the mayonnaise formulation, by decreasing the quantity of yolk pellet, may be expected to produce mayonnaise with optimal consistency.

CONCLUSION

A PROCESS for step-wise recovery of protein and lipid components from egg yolk water-soluble and -insoluble fractions was proposed. It produced value-added products and retained high functionality in the remaining yolk solids which could be used as food ingredients. Since the use of organic solvents was deferred until after recovery of both water-soluble and -insoluble proteins, this new process avoided the possibility of denaturation of proteins by solvents. Therefore, water-soluble proteins such as the immunoglobulin fraction could be recovered for exploitation of biological activity, and the remaining yolk pellet could continue to be used in traditional egg yolk applications. The pellet could be further fractionated to yield components such as proteins and triglycerides for use as food ingredients. Yolk lecithin could be used in food processing as an emulsifier or as a valuable component in preparation of liposomes for drug delivery systems or other medical uses.

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*GROUND CHICKEN MEAT. . .*From page 1528

Any standards for fat content of ground chicken which might be written by a governmental agency and/or poultry industry representations should be designed to inform consumers of product composition and to encourage production of the highest quality products possible. High quality may not necessarily indicate low fat. Our results suggest that levels of fat approaching 20% could be incorporated without serious loss of yield or quality. Higher levels of fat might be useful for controlling product moisture content and texture.

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Emulsifying Salts Influence on Characteristics of Cheese Analogs from Calcium Caseinate

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ABSTRACT

Cheese analogs were prepared from calcium caseinate, butter oil and emulsifying sodium salts (ES). Increasing ES levels gave cheese analogs with higher pH, degree of casein dissociation and degree of fat emulsification than the control without ES. Firmness of cheese analogs first increased, then decreased when the ES level was increased from 1 to 3%. Effects depended on the degree of polymerization of phosphate salts. Sodium citrate (>1%) or Na_2HPO_4 (>2%) made cheese analogs more able to melt upon reheating. Melting ability correlated with high pH, soft texture, high degree of casein dissociation and low degree of fat emulsification.

INTRODUCTION

MANY CHEESE ANALOGS have been prepared with caseinate as the sole or major protein source. According to Chen et al. (1979), the specific melting and rheological properties of such analogs reflect directly those of the constituent caseinate(s). Calcium caseinate was used to produce imitation Mozzarella cheese (IMC) or imitation Cheddar (Wynn et al., 1978). Blends of sodium and calcium caseinates were successfully used to produce IMC suitable for pizza preparation (Petka, 1976; Rule and Werstak, 1978; Rule et al., 1980). A mix of sodium and calcium caseinates was sold (Western Dairy Products Co., trade name SAVORTONE 491 mix). A blend of potassium (25%) and sodium caseinates (75%) was also used (Kasik and Peterson, 1976).

Vegetable proteins were also used in partial or total replacement of caseinate. Chen et al. (1979) reported the effects of incorporating peanut protein isolate. A replacement level of 40–50% gave adequate texture and melting properties. Lee and Marshall (1981) replaced sodium caseinate with native or boiled soy protein concentrate. The hardness and cohesiveness of the resulting curd were reduced. Scanning electron microscopy indicated that the curd containing boiled soy protein was more porus than the control curd or that containing native soy protein. Nishiya et al. (1989a) recently showed that a 40% replacement level of sodium caseinate by soy protein isolate (partly hydrolyzed by enzymes in IMC) improved the melting ability. IMC has also been prepared from lactic acid casein (from sodium caseinate) or from reconstituted calcium paracaseinate (from rennet casein, Nishiya et al., 1989b, 1989c). The cheese prepared from lactic acid casein showed good meltability, whereas IMC from calcium paracaseinate or from sodium caseinate required sodium citrate for adequate meltability. Imitation cheeses may be prepared from pregelatinized or modified high-amylose starch in partial replacement of caseinate (Zwiercan et al., 1986). A mixture of sodium caseinate, soy protein isolate and corn starch has also been used to prepare imitation cheeses (Lee and Son, 1985).

The texture, meltability, color and flavor of cheese analogs should be as close as possible to those of the original cheeses (Siaspentas, 1978). The addition of emulsifying salts may be necessary to impart melting properties. In the manufacture of processed cheeses (which differ from cheese analogs in that cheese is added) emulsifying sodium salts are used to adjust final pH, to chelate and remove calcium ions from protein constituents. As a result, they also disperse, hydrate, and solubilize these protein constituents, which, in turn, enhance fat emulsification and emulsion stabilization. An appropriate structure is thus obtained after cooling (Caric et al., 1985). The effects of emulsifying salts on properties of cheese analogs are less clearly understood. Savello et al. (1989) recently concluded from studies of model cheese analogs prepared with acid or rennent casein that melting properties depended mainly on the type casein used and on the type and concentration of emulsifying salt. In cheese analogs from sodium caseinate, soy protein isolate, soybean oil and corn starch, the melting properties were strongly influenced by proportions of lactic acid and disodium phosphate (Lee and Son, 1985). Hokes et al. (1989) compared the performance of 12 samples of calcium caseinate and found the best cheese emulsions to depend on rapid swelling and particle disintegration plus pseudoplastic behavior over an extended range of shear rates.

In a previous study, we found it possible to prepare cheese analogs by extrusion-cooking of calcium caseinate and butter oil, with or without emulsifying salts. Using a 400-mm-long cooling die attached to the extruder outlet, continuous emulsified and gelled cheese strips were obtained under the following conditions: barrel temperature: 80–120°C; screw speed: 150–200 rpm; feed rate: 30 kg total w/hr; mean residence time: ca 2 min. Compared to batch-prepared cheeses of the same composition, the extruded cheese strips displayed a similar texture, but a somewhat lower degree of fat emulsification and a higher degree of casein dissociation. Addition of emulsifying salts (1% sodium citrate or 2% disodium hydrogen phosphate) in either batch or extruded cheese analogs increased the degree of fat emulsification and imparted melting properties (upon reheating at 230°C for 5 min) (Cavalier et al., 1990).

In our current study, cheese analogs were prepared batchwise from calcium or sodium caseinate with or without different types and concentrations of emulsifying salts. Our objective was to determine the pH, texture, melting characteristics, degree of fat emulsification, degree of casein association/dissociation, and soluble calcium concentration of the resulting cheeses. Relationships between these properties and cheese composition were evaluated.

MATERIALS & METHODS

Materials

Calcium caseinate (95% dry solids; 93.6% total nitrogen; 0.3% nonprotein nitrogen; calcium content: 1.5–1.7 g/100 g dry solids; sodium content: 0.05 g/100 g dry solids; nitrogen solubility index: 84% at pH 6 and 94% at pH 7), sodium caseinate (95% dry solids; 92.9% total nitrogen; 0.5% nonprotein nitrogen; calcium content: 0.15 g/100 g dry solids; sodium content: 1.4–1.7 g/100 g dry solids; nitrogen solubility index: 97% at pH 6 and 99% at pH 7), and butter oil were made and donated by Union Laitière Normande (Condé sur Vire, France). The sodium "emulsifying" salts used were: disodium hydrogen phosphate (dihydrate) and sodium citrate (anhydrous) from Carlo Erba (Milan, Italy); sodium tripolyphosphate (anhydrous) from

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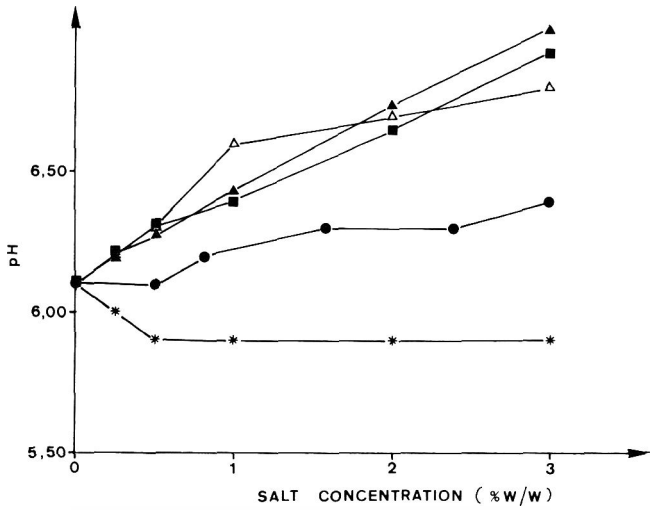


Fig. 1—Influence of concentration of emulsifying salts on pH of cheese analogs: (●) Disodium hydrogen phosphate; (▲) Tetrasodium pyrophosphate; (■) Sodium tripolyphosphate; (*) Sodium polyphosphate; (△) Sodium citrate.

Sigma (St. Louis, MO); sodium pyrophosphate (decahydrate) and sodium polyphosphate (Graham salts) from Merck (Darmstadt, Germany). All chemical reagents were of analytical grade.

Batch preparation of cheese analogs

Cheese analogs were prepared in duplicate using a Stephan Cutter model S12 equipped with a jacketed bowl. The calcium or sodium caseinate powder (about 630 g) was heated to 85°C for 1 min, while mixing at 1500 rpm. Butter oil (400 g), preheated to 75°C, was added and mixed with caseinate at 3000 rpm for 1.5 min. The liquid phase, containing in some cases emulsifying salts or $CaCl_2$, was heated to 80°C before being added to the caseinate-butter oil mix. Emulsification was carried out at 80°C and 1500 rpm during 2 min. About 450 g product was poured hot into 500-mL cylindrical polyethylene containers, cooled at room temperature for 2 hr (final temperature: ca 40°C) then stored at 4°C. Gelation took place upon cooling.

The reported concentration of emulsifying salts refers to each salt without its constitutive water molecules. The cheese analog formulation corresponds to a constant dry solids content of 50% and a constant fat/dry solids weight ratio of 0.4.

Analytical methods

Moisture content was determined by drying 5-g samples 24 hr at 105°C. Determinations were made in triplicate. The coefficient of variation (CV_t), including error due to preparation of samples, was 0.5%. pH of samples was measured with a glass electrode for solid foods.

Cheese samples were equilibrated at 20°C before texture measurement. Firmness was determined as force in Newtons (N) at 3 cm penetration (diameter of probe: 8 mm; flat surface; speed of penetration: 50 mm/min), using an Instron Universal Machine. Results are means of 5 or 6 determinations, and the CV_t = 12%.

Melting characteristics were determined according to the method of Schreiber as described by Park et al. (1984), with some modifications of sample size. Cylindrical cheese samples (ht 5 mm, diam 30 mm) were heated in an oven at 230°C for 5 min. Diameter expansion was measured along four directions and calculated as means of the four directions for five melting tests. Nitrogen content was measured using the Kjeldahl method. Noncasein nitrogen (NCN) was determined on filtrate after mixing 12g of sample with 88g sodium acetate buffer (1 M, pH 4.6), at room temperature.

Nonsedimentable nitrogen (NSN) was determined in duplicate on the supernatant from ultracentrifugation (300,000 x *g*, 1 hr, 20°C) of a dispersion of a 5-g cheese sample in 95 g distilled water (Ultra Turrax, 2 min., 20°C), according to Lee et al. (1979).

The degree of dissociation of caseins was defined as the ratio:

$$\frac{\text{NSN-NCN}}{\text{NT-NCN}} = \frac{\text{NSN}}{\text{NT}}$$

where TN is total nitrogen, and the value for NCN is negligible (<3%). This ratio represents the proportion of caseinate present as casein monomers or small oligomers. The CVt for cheese without emulsifying salt = 9%.

Electrophoresis of protein constituents

This was carried out in urea according to Andrews (1983), with slight modifications. The separating gel (12% acrylamide) contained 4.3 M urea and 0.3 M Tris-0.144 N HCl buffer, pH 8.9. The stacking gel (4% acrylamide) contained 4.3 M urea and 0.062 M Tris-0.144 N HCl buffer, pH 7.6. A 0.024 M Tris-0.19 M glycine buffer, pH 8.3, was used as electrode buffer. About 0.14 mL of the cheese dispersions prepared for the NSN test, before (dispersion A) or after (dispersion B) ultracentrifugation, was diluted with 0.5 mL of 8 M urea containing 2% 2-mercaptoethanol and brought to 1 mL with distilled water (final protein concentration ca 2 mg/mL). Twenty µL was pipetted onto the gel. Electrophoretic patterns from total cheese dispersions (A) indicated the quantitative distribution of α_s, β and κ-caseins. Those from ultracentrifuged cheese dispersions indicated the proportions of each individual casein in the soluble monomeric plus oligomeric fraction. In the case of control α_s, β, or κ-caseins, 20 µL of a dispersion containing 1 mg protein/mL was applied to the gel. Electrophoresis was first performed at 30 W for 10 min, then at 35 W for 4 hr (15°C; 110 V/plate). Gels were stained with a solution of 0.1% Coomassie brilliant blue R in acetic acid/ methanol/ water (10/ 45/45 v/v) for 1 hr at room temperature. Destaining was by washing gels in 7.5% acetic acid in water. The stained bands were quantified with a Model GS 300 densitometer (Hoeffer Scientific Instruments, San Francisco, CA).

Calcium

The calcium content was determined in duplicate by atomic absorption spectrophotometry using a Varian model AA6 apparatus. For total calcium determinations, 1 mL of cheese dispersion A was diluted with 40 mL of a 0.5M sodium citrate solution and with 10 mL of a 0.36M lanthanum oxide dispersion in HCl 9N. This was brought to 100 mL with distilled water (20°C). For "soluble" calcium determinations, 1 mL of ultracentrifugation supernatant (dispersion B), was treated in the same manner. Results were expressed as g soluble calcium/100g total calcium.

Degree of fat emulsification

This was determined by image analysis of scanning electron micrographs (SEM) of cheese samples, prepared according to Kalab and Modler (1985). A Data Sud system (Microscopy Center, University of Montpellier II) equipped with a high-resolution camera was used. Observations were made on the negatives of electron micrographs. The average diameter of fat droplets was measured in duplicate. The CVt for the cheese analog without emulsifying salt = 5.8%.

Statistical evaluations were carried out using the STATITCF (1987) software program from Institut Technique des Céréales et Fourrages, Paris.

RESULTS & DISCUSSION

Influence on pH

The first series of cheese analogs was prepared from calcium caseinate. Effects of different concentrations of emulsifying salts (sodium salts) on final pH are shown in Fig.1. Increasing salt concentrations resulted in higher pH , except with sodium polyphosphates were the final pH was almost unchanged (from 6.1 to 6.0 or 5.9). Addition of 3g of Na_2HPO_4/100 g final cheese analog increased pH from 6.1 to 6.4. However, 3% sodium citrate, sodium pyrophosphate or sodium tripolyphosphate increased pH from 6.1 to 6.8, 7.0 or 6.9, respectively. Repeatability of these effects was checked for cheeses prepared with polyphosphate or Na_2HPO_4 and found to be ± 0.1. The effect of each phosphate salt on pH of cheese analogs was as expected from the pH of a 1% (w/v) aqueous solution of the corresponding salt (Table 1). The pH changes illustrated by Fig. 1 may have been responsible for some of the effects observed in subsequent experiments.

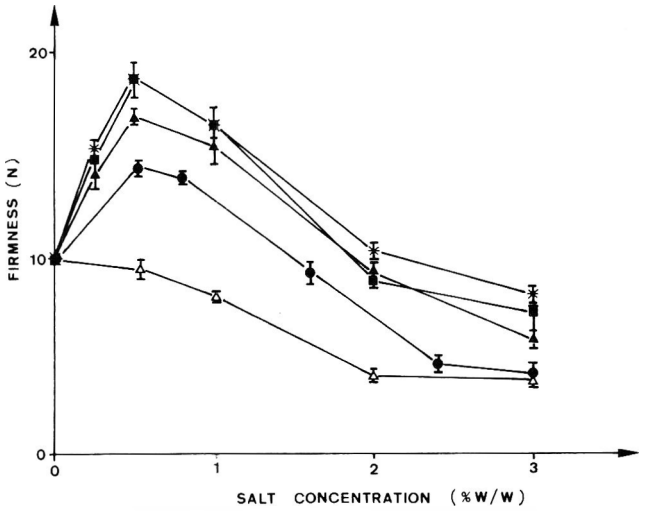


Fig. 2—Influence of concentration of emulsifying salts on firmness of cheese analogs. Firmness determined by penetration: (●) Disodium hydrogen phosphate; (▲) Tetrasodium pyrophosphate; (■) Sodium tripolyphosphate; (*) Sodium polyphosphate; (△) Sodium citrate.

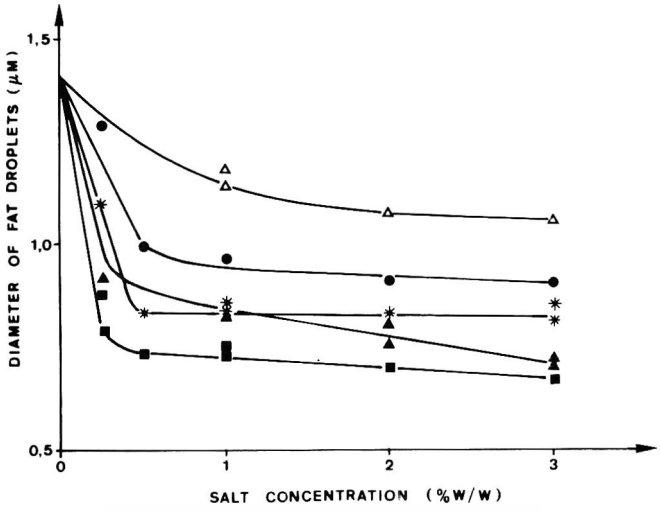


Fig. 3—Influence of concentration of emulsifying salts on mean diameter of fat droplets in cheese analogs. (●) Disodium hydrogen phosphate; (▲) Tetrasodium pyrophosphate; (■) Sodium tripolyphosphate; (*) Sodium polyphosphate; (△) Sodium citrate.

Table 1—Characteristics of emulsifying sodium salts

	pH[a]	Solubility[b] (g/100 mL H_2O) (20°C)	Calcium sequestering power (%)[c]
Na_2HPO_4	9.1	18	33
$Na_4P_2O_7$	10.2	10-12	47
$Na_5P_3O_{10}$	9.3	14-15	82
$(NaPO_3)_n$ (Graham salts)	6.0	very high	87
Sodium citrate	8.2	high	43

[a] as measured, 1% w/v aqueous solution (20°C).
[b] according to Caric et al., 1985.
[c] by solubilization of rennet casein (Heide, 1966)

Table 2—Influence of the concentration of emulsifying salts on melting ability[a] of cheese analogs prepared from calcium caseinate

	g/100 g cheese	Melting diam (mm)
Na_2HPO_4	0	30.0 ± 0.0
	0.25	30.2 ± 2.0
	0.50	30.4 ± 4.8
	1.00	32.8 ± 2.7
	2.00*	41.4 ± 3.7
	3.00*	44.8 ± 2.6
$Na_4P_2O_7$	0.25 to 2.00	30.0 ± 0.0
	3.00	30.5 ± 2.5
$Na_5P_3O_{10}$	0.25 to 1.00	30.0 ± 0.0
	2.00	31.0 ± 4.0
	3.00	31.5 ± 5.0
$(NaPO_3)_n$	0.25 to 3.00	30.0 ± 0.0
Sodium citrate	0.25	36.7 ± 8.4
	0.50	39.8 ± 10.0
	1.00*	42.1 ± 4.0
	2.00*	51.0 ± 3.0
	3.00*	54.0 ± 2.1

[a] mean sample diameter (mm) after heating (230°C; 5 min). Sample diameter before heating was 30 mm.
* significantly different from that prepared without emulsifying salt ($p > 10\%$).

Influence on firmness and melting properties

Addition of each of the emulsifying salts in a range 0 to 0.5% w/w increased firmness, except with sodium citrate (Fig. 2). At higher salt concentrations (1–3%), firmness decreased. The maximum increase in firmness was observed for phosphate salts with a high degree of polymerization at 0.5% w/w. These data are in agreement with those of Swiatek (1964), who studied the effects of sodium citrate and phosphates on texture of processed cheese. Disodium phosphate and sodium citrate produced similar soft cheeses, and cheese firmness increased with degree of phosphate polymerization.

The melting properties (upon reheating) are shown in Table 2. Sodium pyrophosphate, tripolyphosphate or polyphosphate did not induce heat-melting, in contrast to 1% sodium citrate or 2% disodium phosphate. Similar results have been reported for processed cheeses. Thomas et al. (1980) showed that disodium phosphate led to better melting properties than sodium pyrophosphate or tripolyphosphate. Gupta et al. (1984) reported that sodium citrate increased the melting ability better than sodium phosphates. In our study, firmness and melting ability were inversely related, since the cheese analogs with sodium citrate or disodium phosphate had both the softest texture and the highest melting ability. Taneya et al. (1980) prepared a soft processed cheese that contained 43% moisture; 27% fat, 1.5% sodium polyphosphate and 1% sodium citrate, which melted upon reheating. In contrast, a hard-type processed cheese obtained with 44% moisture, 26% fat, 2.2% sodium polyphosphate and no sodium citrate did not melt. Kalab et al. (1987) studied the microstructure and texture properties of processed cheeses. A negative correlation was observed between cheese firmness and the melted diameter after reheating at 140°C for 6 min.

In our cheese analogs, a soft texture appears necessary, but not always sufficient, in order to impart melting ability upon reheating. For example, cheese analogs prepared with 3% sodium polyphosphate had a soft texture (firmness below 8N), but did not melt upon reheating. It is thus likely that the degree of fat emulsification, the degree of casein dissociation, and the pH of the cheese analogs also influence melting (dry solids and fat content remained constant).

Influence on degree of fat emulsification

The degree of fat emulsification was determined by scanning electron microscopy, and image analysis as indicated (Fig. 3). The addition of any of the emulsifying (sodium) salts in the concentration range 0.2- 3.0% resulted in a considerably smaller diameter of fat droplets. The maximum effect was obtained at a 0.5% salt concentration. The effect was dependent on type of salt, in the following decreasing order (at 1% salt concentration): tripolyphosphate > pyrophosphate = polyphosphate > disodium hydrogen phosphate > sodium citrate. The emul-

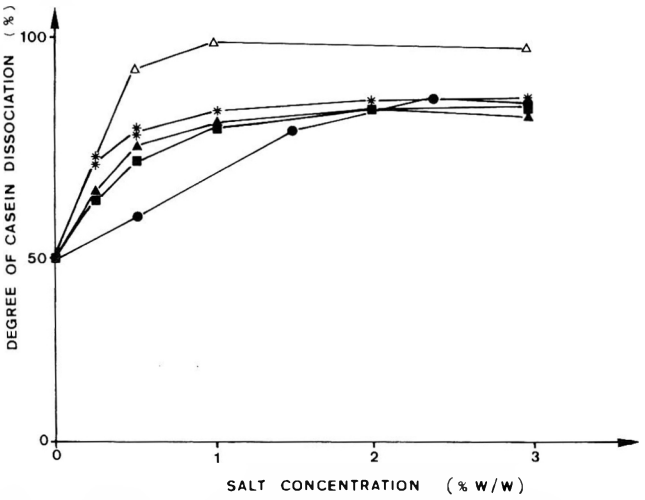


Fig. 4—Influence of concentration of emulsifying salts on degree of casein dissociation of cheese analogs: (●) Disodium hydrogen phosphate; (▲) Tetrasodium pyrophosphate; (■) Sodium tripolyphosphate; (*) Sodium polyphosphate; (△) Sodium citrate.

sifying ability of these sodium salts in processed cheese systems is well known. By chelating and removing calcium from protein constituents, these salts convert calcium paracaseinate into soluble dissociated sodium caseinate with fat emulsifying properties (Caric et al., 1985). This mechanism does not necessarily apply with cheese analogs. Less finely emulsified cheese analogs usually display better melting properties (Shimp, 1985). Rayan et al. (1980) reported an inverse relationship between fat emulsification and melting ability of processed cheese upon reheating, but no quantitative data were reported. According to Hokes (1982), close interactions between fat and the hydrophobic zones of proteins may prevent melting.

The increasing degree of fat emulsification we observed when emulsifying (sodium) salts were added at 0 to 0.5% may in turn have been responsible for increasing firmness of cheese analogs. However, the decrease in firmness noted when concentrations increased from 0.5 to 3% (Fig. 2) was not related to the diameter of fat droplets since this diameter remained constant above 0.5% emulsifying salt (Fig. 3). The lesser firmness may have been due to a looser protein matrix, as a result of increasing pH and possible calcium release from protein constituents.

Influence on protein matrix

The degree of casein dissociation, as determined by the proportion of soluble caseinate not sedimentable by ultracentrifugation, was near 50% in cheese analogs prepared without emulsifying salt (Fig. 4). Casein dissociation increased with both concentration of emulsifying salt and degree of polymerization of the phosphate salts. Note that increasing pH values, brought about by higher concentrations of the salts, may not fully account for the higher degree of casein dissociation. Control cheese analogs adjusted to pH 6.4 or 6.6 with NaOH without emulsifying salt had lower degrees of casein dissociation (55 or 75%) than those prepared with emulsifying salts at the same pH (≥76 or ≥85%) (Fig. 4) Therefore it is likely that high degrees of casein dissociation depend both on higher pH and calcium complexation caused by the emulsifying salts. Lee (1981) observed similar effects with processed cheeses from calcium paracaseinate. The degree of casein dissociation is probably related to the affinity of the emulsifying anion for calcium, since destruction of protein-calcium-protein interactions reduces aggregation. In our experiments, the degree of casein dissociation of cheese analogs containing 3% of any phosphate salt reached a maximum of 84-86% (Fig. 4). Sodium citrate always led to a higher degree of casein dissociation than phosphate salts (at equal salt concentrations). Thus citrate appeared to chelate calcium ions effectively (in contrast to reports by von der Heide, 1966, Table 1).

Since the binding of calcium to caseinate depends on the degree of phosphorylation of the casein, the addition of different calcium complexing salts may affect differently the solubility of different caseins. The electrophoretic patterns of total cheese extracts (A), as determined in the presence of urea, were therefore compared (for analogs with different salts) to those of corresponding supernatants (B) from ultracentrifuged total cheese extracts (A) (Fig.5). The comparison showed that the soluble but sedimentable (300,000 x *g*; 1 hr) casein aggregates (that probably depend on calcium-protein interactions) displayed the highest α_s/β casein ratio when cheese analogs contained 1% sodium citrate or 0.5% tetrasodium pyrophosphate.

We noted that κ-casein gave a diffuse electrophoretic pattern, and that sodium tripolyphosphate caused insoluble protein

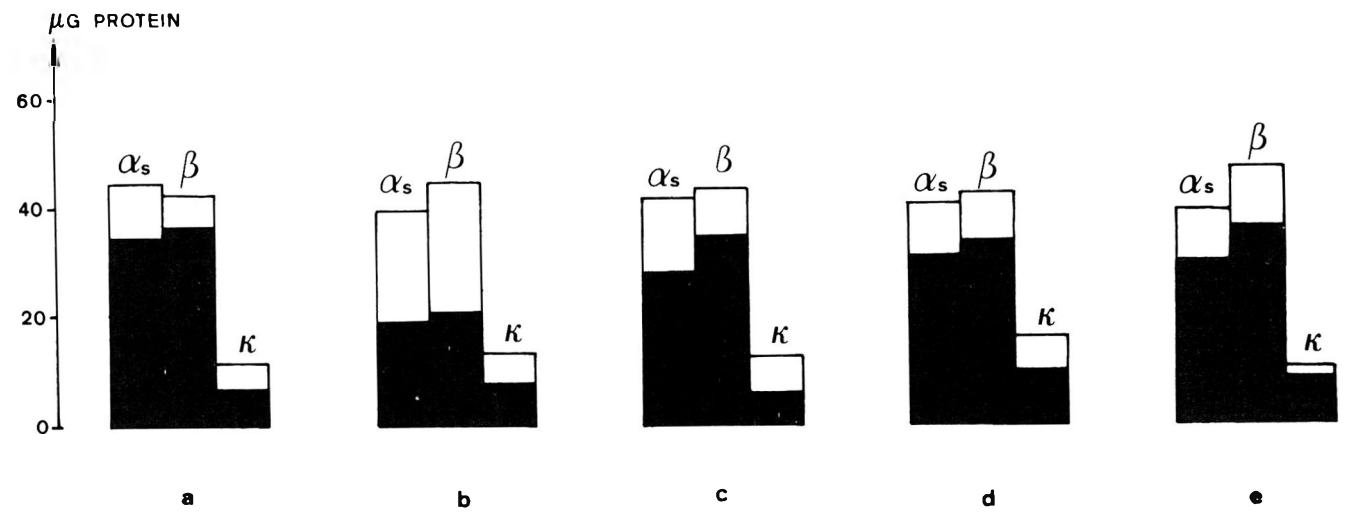


Fig. 5—Electrophoretic patterns of soluble cheese extracts (PAGE, urea, mercaptoethanol). Expressed as μg protein/ 100 μg protein in total soluble cheese extracts. Densitometry carried out after staining with Coomassie brilliant blue R. □ Total soluble cheese extract; ■ Supernatant after ultracentrifugation (300,000 x g, 1 hr) of soluble cheese extracts. (a) 1% sodium citrate;-(b) 0.5 disodium hyrdogen phosphate; (c) 0.5% tetrasodium pyrophosphate; (d) 0.5% sodium tripolyphosphate; (e) 0.5% sodium polyphosphate.

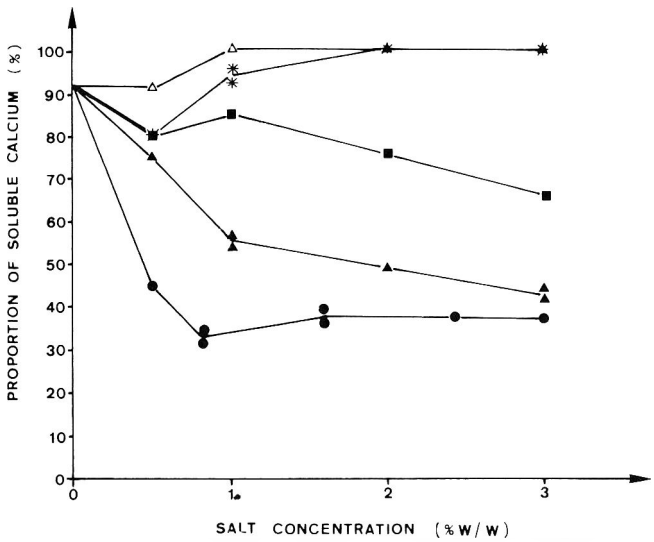


Fig. 6—Influence of concentration of emulsifying salts on soluble calcium content of cheese analogs: (●) Disodium hydrogen phosphate; (▲) Tetrasodium pyrophosphate; (■) Sodium tripolyphosphate; (*) Sodium polyphosphate; (△) Sodium citrate.

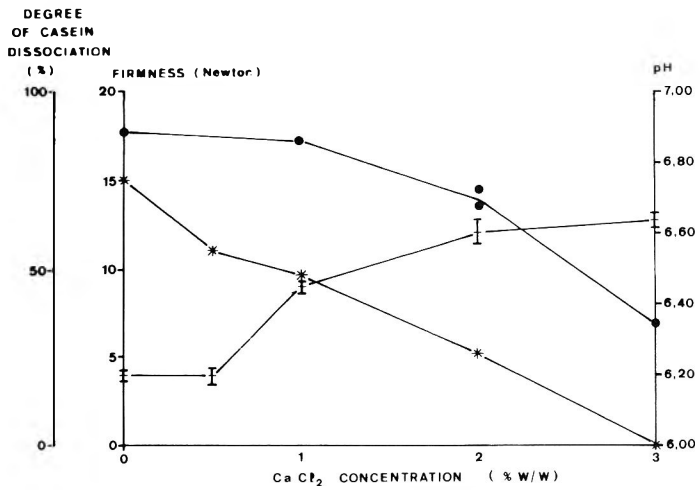


Fig. 7—Influence of concentration of $CaCl_2$ on firmness, pH and degree of casein dissociation of cheese analogs prepared from sodium caseinate: (†) firmness (N); (*) pH; (●) degree of casein dissociation (%).

fractions to remain in the stacking gel. In spite of those difficulties, the degree of casein dissociation could be calculated from the electrophoretic patterns of Fig. 5. The values thus obtained, 80% (a), 49% (b), 70% (c), 75% (d), 79% (e) were quite similar to those determined through total protein solubility (Fig. 4).

Influence on soluble calcium

The proportion of calcium that was not sedimented after ultracentrifugation (300,000 x *g*, 1 hr) was high (90–94%) with the analog prepared without emulsifying salt. The addition of most phosphate emulsifying salts (except polyphosphate) decreased the proportion of soluble calcium. However, this depended on the type and concentration of emulsifying salt (Fig. 6). The decrease in soluble calcium was observed over the salt concentration range (0.5–3.0%) with sodium monophosphate or sodium pyrophosphate and to a lesser extent with sodium tripolyphosphate. In contrast, the proportion of soluble calcium remained high (85–100%) at all concentrations of sodium polyphosphate or sodium citrate.

Table 3—Calcium and sodium concentrations[a] and melting ability[b] of cheese analogs prepared from calcium caseinate or from sodium caseinate with or without emulsifying salts or $CaCl_2$

Composition	Ca[a]	Na[a]	Ca/Na (molar ratio)	Melting ability[b]
Calcium caseinate	11.7	2.3	5.08	–
Calcium caseinate + 1% sodium citrate	11.7	14.0	0.83	+
Calcium caseinate + 2% Na_2HPO_4	11.7	16.3	0.72	+
Sodium caseinate	1.12	20.0	0.06	+
Sodium caseinate + 0.5% $CaCl_2$	5.62	20.0	0.28	+
Sodium caseinate + 1% $CaCl_2$	10.1	20.0	0.51	+
Sodium caseinate + 2% $CaCl_2$	19.1	20.0	0.95	–

[a] as expressed in mmole of Ca or Na/100 g cheese analog.
[b] melting ability measured at 230°C for 5 min. + = melts.

These results are tentatively interpreted as follows. In the cheese analog prepared without emulsifying salt, the high proportion of soluble calcium may have been due to soluble calcium salts (or dissociated ions) and to calcium bound to soluble (i.e., nonsedimentable) protein constituents. In the presence of sodium polyphosphate or sodium citrate, additional calcium may have been converted into soluble calcium salts. The amount of calcium bound to soluble protein constituents may have also increased since the degree of casein dissociation was found to increase (Fig. 4). New calcium salts may have also formed and bound to protein constituents without insolubilizing them. In the presence of sodium mono- and pyrophosphates, insoluble calcium phosphates may have been formed.

The calcium content of the cheese analogs can be calculated from that of the calcium caseinate, 0.45g (11.2 mmole)/100g cheese analog. When added at 1% (w/w) the content of the various emulsifying salts was 3.9 mmole (citrate), 7.0 mmole (disodium hydrogen phosphate), 3.7 mmole (sodium pyrophosphate) and 2.4 mmole (sodium tripolyphosphate)/100g cheese analog.

Characteristics of cheese analogs from sodium caseinate with or without calcium chloride

When the cheese analog was prepared from sodium caseinate in place of calcium caseinate, the following characteristics were noted: higher pH (6.7 vs 6.1); lower firmness (4 N vs 9.2 N); higher degree of fat emulsification (avg diam of fat droplets: 0.64 μm vs 1.37 μm); higher degree of casein dissociation (88% vs 50%); melting ability (Fig. 7 and 8).

Cheese analogs were also prepared from sodium caseinate in the presence of increasing concentrations of $CaCl_2$ (0.5–3%, corresponding to 4.5–27 mmole Ca/100g cheese analog). The resulting characteristics are shown in Fig. 7 and 8. Firmness increased with $CaCl_2$ concentration (4N to 12N). This increase may have been due to the corresponding decrease in pH (from 6.75 to 6.0). However, the firmness of cheese analogs prepared from sodium caseinate with pH adjustment without addition of $CaCl_2$ did not vary as extensively (from 4.0 ± 0.5 Newtons at pH 6.75 to 6.0 ± 0.4 Newtons at pH 6.25).

Increasing concentrations of $CaCl_2$ caused lower degrees of casein dissociation (Fig. 7). This was probably due to protein-calcium interactions. In parallel, increasing concentrations of $CaCl_2$ from 0 to 1% resulted in a higher degree of fat emulsification (Fig. 8). Since the degree of casein dissociation remained constant and the pH was somewhat decreased, the degree of fat emulsification seemed related to an optimal Ca/ Na ratio. At higher $CaCl_2$ concentrations (1–3%), the degree of fat emulsification decreased, probably because the emulsifying properties of caseinate were reduced at lower caseinate solubility. However its solubility remained higher than in the cheese an-

Table 4—Correlations between melting ability and other characteristics of cheese analogs[a] prepared from calcium caseinate

	Firmness	Degree of fat emulsification	Degree of casein dissociation	Proportion of soluble calcium	pH
Na_2HPO_4					
r^b =	−0,79	−0,80	0,98*	−0,25	0,98*
Sodium citrate					
r^b =	−0,95*	−0,98*	0,98*	0,74	0,95*

[a] Cheese analogs prepared with 0-3% disodium hydrogen phosphate or with 0-3% sodium citrate; dry solids: 50%; fat content: 40% dry basis.
[b] r = linear correlation coefficient
*p≤0.01

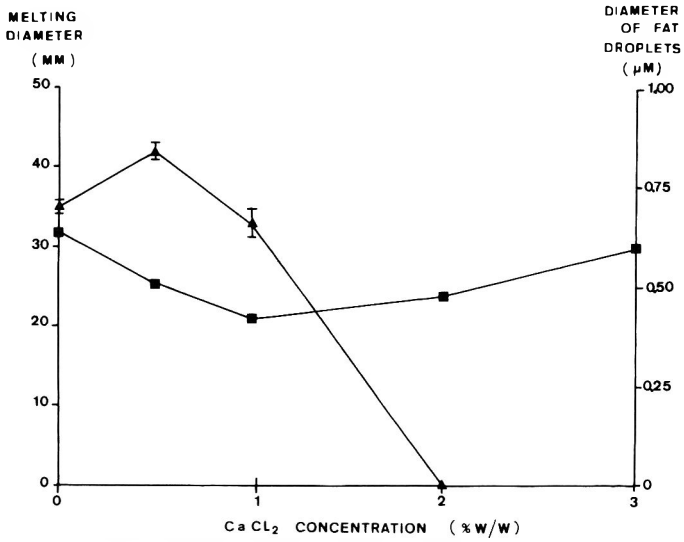


Fig. 8—Influence of concentration of $CaCl_2$ on diameter of fat droplets and melting ability of cheese analogs prepared from sodium caseinate: (■) diameter of fat droplets (μm); (▲) melting diameter (mm).

alog prepared from calcium caseinate (without emulsifying salt) (Fig. 3).

The high melting ability of the cheese analog prepared with sodium caseinate remained unchanged when 0.5 or 1% $CaCl_2$ was added (Fig. 8). However, the melting ability was lost when 2% of $CaCl_2$ (or more) was added (19 mmole Ca/100g cheese, Ca/Na molar ratio of 0.95, Table 3). The Ca/Na molar ratio at which the melting ability was lost was > 0.95 for the cheese analogs prepared from sodium caseinate > 0.83 for those from calcium caseinate. These two types of analogs were also similar with respect to firmness, pH, and degree of casein dissociation, but not to degree of fat emulsification or stickiness. The cheese analogs prepared from sodium caseinate were unacceptably sticky, but this diminished upon addition of 1% $CaCl_2$.

Correlation between melting ability and other characteristics

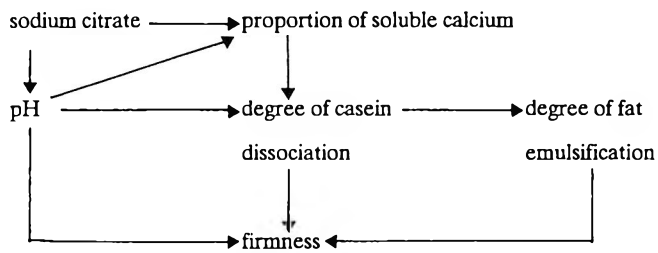
The main characteristics of the cheese analogs from calcium caseinate were analyzed statistically for possible correlations among those characteristics and between some characteristics and type/ concentration of emulsifying salt. Melting ability highly correlated ($|r| = 0.98$) with degree of casein dissociation (Table 4) and pH of the analogs ($|r| = 0.95$). In addition, melting ability negatively correlated with firmness ($|r| \geq 0.79$) and degree of fat emulsification ($|r| \geq 0.80$). As previously discussed, the comparison of cheese analogs from calcium caseinate (with or without sodium emulsifying salts) and from sodium caseinate (with $CaCl_2$ added) indicated that the melting ability also depended on a low Ca^{2+}/Na^+ molecular ratio (ca 0.95).

Addition of sodium citrate (0.25–3%, w/w) to cheese analogs prepared from calcium caseinate can be used as an example. Since firmness, degree of casein dissociation, degree of fat emulsification and pH highly correlated, and since soluble calcium correlated with degree of casein dissociation, and pH (Table 5), possibly we could assume the following cause-and-effect relationships:

Table 5—Linear regression coefficients (r) between different characteristics of cheese analogs[a] prepared from calcium caseinate and sodium citrate

	Firmness	Degree of casein dissociation	Degree of fat emulsification	Proportion of soluble calcium	pH	Melting ability
Firmness	1.00					
Degree of casein dissociation	0.97**	1.00				
Degree of fat emulsification	0.92**	−0.98**	1.00			
Proportion of soluble calcium	0.80	0.84*	0.79	1.00		
pH	0.92**	0.98**	0.98**	0.89*	1.00	
Melting ability	−0.95**	0.97**	−0.98**	0.74	0.95**	1.00

[a] Cheese analogs prepared with 0-3% sodium citrate; dry solids: 50%; fat content: 40% dry basis.
* p≤.05
** p≤.01



Such a scheme is applicable also for disodium hydrogen phosphate, but no significant correlation was found between proportion of soluble calcium and degree of casein dissociation. With other emulsifying salts, the relationships were less clear. The only significant correlation concerned the degree of casein dissociation and the degree of fat emulsification ($r \geq 0.83$). These various relationships did not apply to the cheese analogs prepared from sodium caseinate.

Savello et al. (1988) have shown that the melting ability of a model cheese analog was affected both by type and concentration of emulsifying salt and by the nature of casein used. Fleming et al. (1985) analyzed two commercial calcium caseinates and found they varied in calcium, γ-casein and free amino group content, water absorption capacity and stability to added calcium. It is therefore likely that such commercial caseinates would also behave differently when used for the manufacture of cheese analogs.

CONCLUSIONS

CHEESE ANALOGS prepared from calcium caseinate were able to melt upon reheating, provided they contained sodium citrate (1%) or disodium hydrogen phosphate (2%). This melting ability appeared to be linked to high pH, high degree of casein dissociation, relatively low degree of fat emulsification, and consequently low firmness. Such cheese analogs could be used for pizza, hot sandwiches, hamburgers or pasta and other cheese-containing dishes.

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—Continued on page 1551

Average NaCl Concentration in Cheese for Different Volume Ratios of Brine and Solid during Salting

SUSANA E. ZORRILLA and AMELIA C. RUBIOLO

ABSTRACT

Fick's second law for unsteady-state, one-dimensional diffusion in solid media from a well stirred solution of limited volume was used to develop mathematical equations for modeling the mass transfer of NaCl in cheeses durirg salting. A computational method was employed to solve the equations and analyze the suitable conditions for salt penetration in finite cylindrical cheeses. Various volume ratios of solution and solid were considered to determine the variation of NaCl concentration in cheeses. Experimental results were predicted by the proposed mathematical model with less than 10% error.

Key Words: sodium chloride, brine, cheese, volume-ratio, solids

INTRODUCTION

THE DIFFUSION and distribution of salt during brining and ripening are important processes in cheese production. The effect of salt on cheese composition has been thoroughly studied. Geurts et al. (1974, 1980) analyzed the total salt uptake for several kinds of brined cheeses under different conditions. Guinee and Fox (1986a,b) studied the effect of geometry on the movement of sodium chloride during brining. They reported how salt absorption changed with fermentation of residual lactose, proteolysis and lipolysis of milk proteins and fats.

Although an appropriate ratio of the volume of the NaCl solution and cheese is desirable for reducing salting time and processing cost, the expression for a quantitative balance has not yet been established. Equations to determine NaCl concentration in cheeses have been presented for an infinite brine volume. Average salt concentration is used to indicate the concentration value in the cheese after uniform distribution. The average salt content vs time changes for different volume ratios of solution and solid during brining. Our objective was to develop equations to show the necessary brining time to reach a desired average salt concentration after defining the design variables. Conditions for a suitable volume of brine were obtained using computational calculus. Experiments were carried out after selecting the required conditions in order to test the predicted values.

THEORY

THE CHEESE was assumed to be a finite cylinder of radius r and height h immersed in a NaCl solution of limited volume which occupied the space of radius R and height H. The mathematical solutions were obtained after considering constant NaCl diffusion coefficient D and uniform initial NaCl concentration in the cheese (roughly zero), and uniform NaCl concentration C_o in the solution.

The solution of the partial differential equation for a finite body can be obtained as a "product-type solution" for certain types of initial and boundary conditions (Carslaw and Jaeger, 1959). The product of the one-variable infinite solutions, expressed as concentration fractions that still can be taken up, can be shown to satisfy the differential equation and conditions for the diffusion of solutes in a finite cylinder from a stirred solution of limited volume. That is, it is necessary to study the solutions for infinite solids associated with this type of geometry.

The solutions for diffusion in an infinite solid from a stirred solution of limited volume were given by Crank (1975). The infinite cylinder solution expressing the total amount of solute M_t in the solid at time t as a fraction of M_∞, the corresponding quantity after infinite time, is

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{4\alpha(1+\alpha)}{4+4\alpha+\alpha^2 q_n^2} \exp\left(-D\, q_n^2\, t/r^2\right) \quad (1)$$

The q_n's are the nonzero positive roots of

$$\alpha\, q_n\, J_o(q_n) + 2\, J_1(q_n) = 0 \quad (2)$$

where $J_o(x)$ and $J_1(x)$ are the Bessel functions of the first kind of zero order and first order, respectively. α is the volume ratio of solution and solid. Considering a partition factor K between solute in the cylinder in equilibrium with the solute in the solution, α is defined as

$$\alpha = \frac{R^2 - r^2}{r^2\, K} \quad (3)$$

Similarly, the solution for the infinite slab is:

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\beta(1+\beta)}{1+\beta+\beta^2 p_n^2} \exp\left(-4\, D\, p_n^2\, t/h^2\right) \quad (4)$$

where p_n's are the nonzero positive roots of

$$\tan(p_n) = -\beta\, p_n \quad (5)$$

β, the volume ratio of solution and slab including K, is defined as

$$\beta = \frac{H - h}{h\, K} \quad (6)$$

The solution for finite cylinder (fc) geometry is obtained as the product of infinite cylinder (c) and infinite slab (s) solutions in the appropriate arrangement that satisfies the conditions of "product type solutions" (Schwartzberg, 1980):

$$\left(1 - \frac{M_t}{M_\infty}\right)_{fc} = \left(1 - \frac{M_t}{M_\infty}\right)_c \left(1 - \frac{M_t}{M_\infty}\right)_s \quad (7)$$

The volume ratio of solution and solid for finite cylinder geometry considering K can be defined as:

$$\gamma = \frac{HR^2 - hr^2}{h\, r^2 K} \quad (8)$$

α and β must be obtained from γ for the finite case. In order to simulate the pure radial diffusion, the plane surfaces of the cylinder are supposedly impermeable. In this case, the cylinder and solution heights are not infinite and $\alpha = \gamma$. Similarly, if the cylindrical surface is supposedly impermeable, the diffusion takes place in the flat sides and neither r nor R are infinite, then $\beta = \gamma$. Eq. (7) can be written as

$$\frac{M_t}{M_\infty} = 1 - \left(\sum_{n=1}^{\infty} \frac{4\gamma(1+\gamma)}{4+4\gamma+\gamma^2 q_n^2} \exp\left(-Dq_n^2 t/r^2\right)\right) \left(\sum_{n=1}^{\infty} \frac{2\gamma(1+\gamma)}{1+\gamma+\gamma^2 p_n^2} \exp\left(-4Dp_n^2 t/h^2\right)\right) \quad (9)$$

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Table 1—Initial composition of cheeses

Component	Average value (% w/w)	SD
Protein	30.156	0.076
Fat	17.461	0.271
Moisture	49.355	0.010
NaCl	–	–

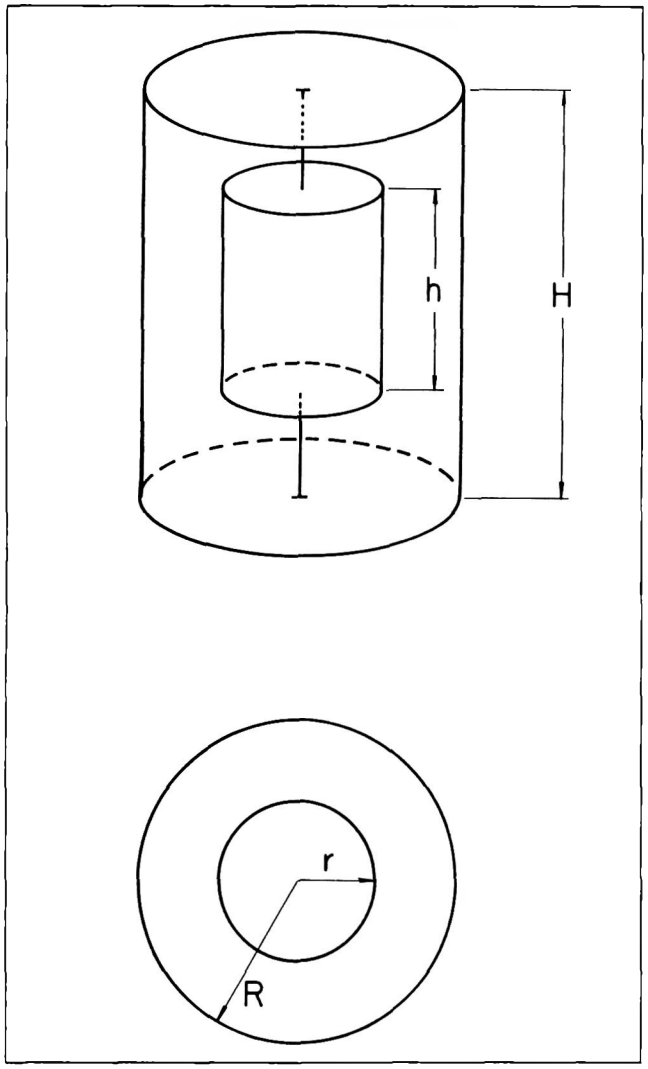

Fig. 1—Schematic of salting device.

Total M_t/M_∞ can be obtained using Eq. (2), Eq. (5), Eq. (8), and Eq. (9).

Although M_∞ is a very common reference for expresssing the results, the effects of changing the solution volume is immediate and easily observed using a constant reference value as C_o. M_∞ can be expressed in terms of γ, C_o, and the volume of solution, using a mass balance. For finite cylinder geometry the following equation is obtained:

$$M_\infty = \frac{\pi (HR^2 - hr^2) C_o}{1+\gamma} \quad (10)$$

M_t in the cylinder after time t is expressed as:

$$M_t = \pi r^2 h X_t \quad (11)$$

where X_t is the average NaCl concentration in the cheese. The ratio of X_t and C_o is finally obtained using Eq. (8), Eq. (10), and Eq. (11).

$$\frac{X_t}{C_o} = \frac{M_t}{M_\infty} \frac{\gamma K}{(1+\gamma)} \quad (12)$$

Table 2—Experimental data of X_t, C_o, W at different times (Rv=5)

Assay	Salting time(hr)	X_t(g/L)	W(%w/w)	C_o(g/L)
1	8	19.07	47.85	250.40
2	10	19.48	47.52	246.92
3	12	21.78	47.61	244.50
4	14	23.03	46.76	254.18
5	16	27.69	46.87	254.98
6	18	29.42	46.80	253.83

Table 3—Corrected experimental data of X_t and C_o at different times (Rv=5)

Assay	Salting time(hr)	X_t(g/L)	C_o(g/L)
1	8	19.67	230.31
2	10	20.23	227.11
3	12	22.58	224.88
4	14	24.30	233.79
5	16	29.15	234.52
6	18	31.03	233.47

MATERIALS & METHODS

FYMBO CHEESES used in the experimental assays were from a commercial factory before salting and transported to the laboratory in a plastic vacuum bag. The cylindrical cheese (7.5 cm radius × 7 cm height) was cut with a sharp cutting edge of a cylindrical mold, to obtain the uniform central part of 6 cm radius and 6 cm height. Several cheese samples were taken to determine the initial composition. These results are shown in Table 1.

The cheeses were brined at different times using a 20% NaCl solution, at 12°C and 88% relative humidity, with 0.55% Ca and pH 5 (Geurts et al., 1974). The brine volume was 5 × larger than the cheese volume in order to be in agreement with characteristic parameters for semi-hard cheeses ($D = 3.024\ 10^{-6}$ cm²/sec, $K = 0.427$, $X_\infty = 85.842$ g/L, $W_\infty = 38.08\%$) given by De Piante et al. (1989); an analogous value of a diffusion coefficient for moisture in cheese was obtained from Geurts et al. (1974) for the same cheese moisture and fat content. Agitation was provided by an air diffuser. The cheese arrangement in the solution is shown in Fig. 1.

Each cheese was taken out of the salting solution after a specified time, dried with paper and assayed for NaCl and moisture content. NaCl content was estimated by Cl^- titration (Fox, 1963) with an automatic titrator (Mettler DL 40RC). Moisture content W was determined using a CEM microwave oven. Protein was determined (N × 6.38) with a Buchi 430-322 apparatus and fat content was estimated using the Standard International Dairy Federation method (SA:1961).

RESULTS & DISCUSSION

EXPERIMENTAL RESULTS are shown in Table 2. Due to the water movement out at the same time that salt penetrates into cheese, resulting in a net weight loss, a correction of X_t values was considered by calculating the salt concentration at the initial moisture content W_o. C_o was also readjusted to an apparent concentration when the dilution effect of the out coming water was included. This correction was done using W_o and W_∞ to estimate the water volume added to the solution for cheese water loss. The corrected X_t and C_0 are given in Table 3.

In order to handle a simpler parameter than γ, it is convenient to use Rv, the volume ratio of solution and cylinder without considering K, therefore $\gamma = Rv/K$.

The percent deviation was estimated with:

$$\% \text{ deviation} = \frac{SD_m}{Y} \times 100$$

where the standard deviation on the model SD_m is

$$SD_m = \sqrt{\frac{\sum_{n=1}^{N} (y - Yt)^2}{N-1}}$$

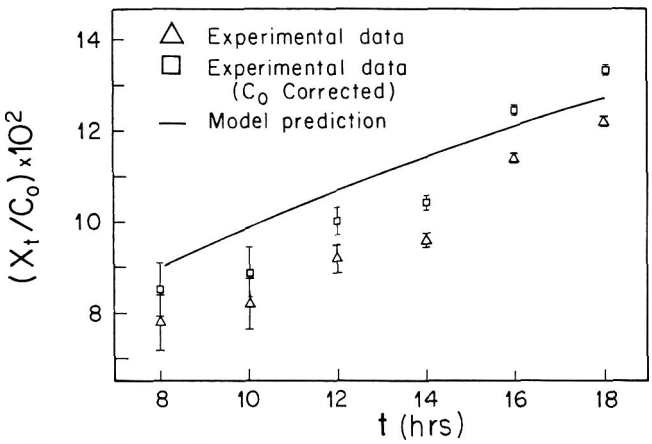

Fig. 2—Experimental and theoretical values of total X_t/C_o at different times (Rv = 5).

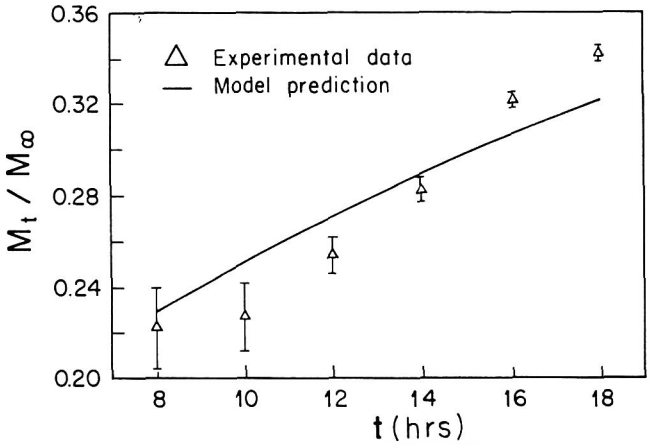

Fig. 3—Experimental and theoretical values of total M_t/M_∞ at different times (Rv = 5).

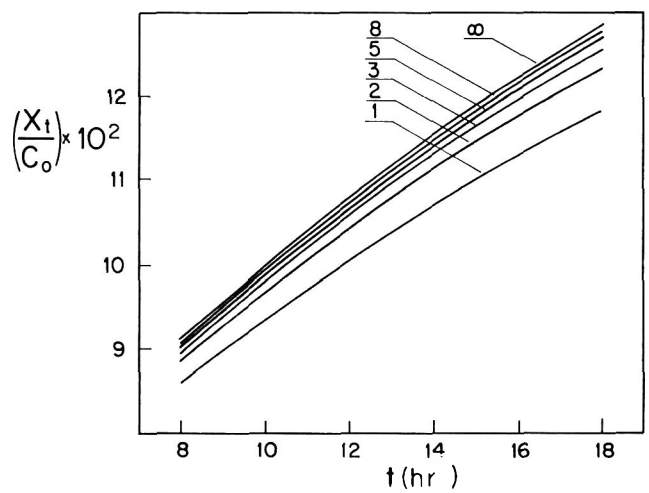

Fig. 4—Effect of Rv on the relation X_t/C_o.

Y is the experimental value, Yt is the theoretical value, and N is the number of points considered.

Figure 2 shows the results of measuring X_t/C_o for C_o given in Table 2 and 3 and the theoretical values predicted by the mathematical model for Rv = 5. In the range analyzed the average percent deviation was 14.2% for C_o without correction and 7.70% when C_o was corrected. Although the first case had

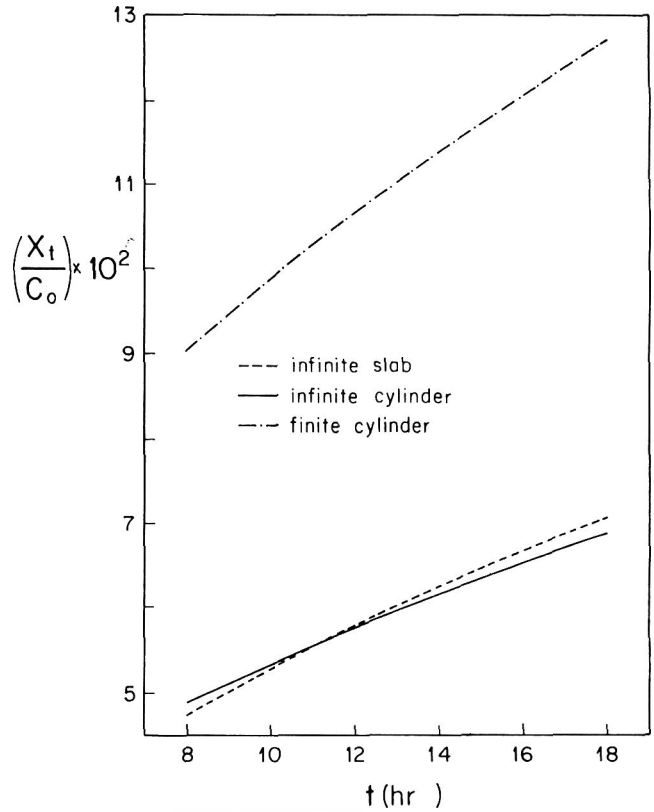

Fig. 5—Radial and plane diffusion contributions to the finite cylinder concentration (Rv = 5).

a higher average percent deviation, it is useful when concentration values at infinite time are not available and only initial concentration of solution is known. The relation M_t/M_∞ vs t is shown in Fig. 3. The average percent deviation was 6.7%. Neither M_t nor M_∞ have been corrected because these variables were associated with the same correction for the water movement out.

The equations were evaluated by a computational method. The relation X_t/C_o vs t was plotted for many volume ratios of solution and solid. Figure 4 shows that, for a given time, after a certain Rv the salt concentration in the cheese did not increase. In that case, the increment in the concentration ratio X_t/C_o was small while the volume ratio increased. Thus an increase of Rv would not always be convenient for the process cost. It is still possible to achieve a degree of equilibrium between process cost and desirable salting values. If Rv = 5 is considered infinite, the error in the calculations was not appreciable, because the curves for Rv greater than 5 were near each other. Figure 5 shows the radial and plane contribution to the total amount of the incorporated salt for Rv = 5.

CONCLUSIONS

THE OPERATING CONDITIONS for salting may be obtained quickly with this mathematical model, giving the appropriate volume of brine and the time to reach the desired salt concentration. This mathematical formulation showed that the total salt uptake was only dependent on Rv and total solid volume. The fitting of equations to experimental data was acceptable. A useful relation for easy estimation of the average NaCl concentration in cheese is given by the ratio X_t/C_o. Results may be estimated even when C_o cannot be corrected taking account of water loss during salting. However, larger average percent deviation is then obtained. X_t/C_o may be estimated reducing the average percent deviation to about 7% when experimental data at infinite time are available. These data must be known

to estimate M_t/M_∞. In that case, the average percent deviation is 6.7%.

NOMENCLATURE

C	NaCl concentration in the solution, g/L
D	diffusion coefficient, sq cm/sec
h	height of the cheese, cm
H	height of the solution, cm
J_0	Bessel function of zero order
J_1	Bessel function of first order
K	partition coefficient
M	mass of the solute in the cheese, g
N	number of experimental data points
p	nonzero root of Eq. (5)
q	nonzero root of Eq. (2)
r	radius of the cheese, cm
R	radius of the solution, cm
Rv	volume ratio of solution and cylinder
SD	standard deviation
SD_m	standard deviation on the model
t	time, sec
W	moisture content of the cheese, % w/w
X	average NaCl concentration in the cheese, g/L
Y	experimental values
Yt	theoretical values
α	relation defined by Eq. (3)
β	relation defined by Eq. (6)
γ	relation defined by Eq. (8)

Sub indices

o	initial
c	infinite cylinder
fc	finite cylinder
n	series terms
s	infinite slab
t	at time t
∞	at infinite time

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Sodium Caseinate from Skim Milk Powder by Extrusion Processing: Physicochemical and Functional Properties

V.L. BARRAQUIO and F.R. VAN DE VOORT

ABSTRACT

Sodium caseinate produced on a pilot-plant-scale directly from skim milk powder (SMP) with two-step extrusion process was compared to a commercial product and a sodium caseinate prepared from commercial acid casein by extrusion-processing. The products were compared in terms of physicochemical properties and functional properties, as well as microbiological quality. Although differences existed between the caseinates, overall, those produced from SMP by extrusion appeared comparable to commercial caseinate. Results indicate that a good-quality sodium caseinate with the requisite properties normally associated with commercial sodium caseinate could be produced directly from SMP by extrusion-processing.

INTRODUCTION

FARM MILK overproduction has been a recurrent problem in Canada, the United States and the European Economic Community. Much of this milk goes into the manufacture of skim milk powder (SMP). When the world SMP supply is in excess, competition to market the surplus results in depressed prices. A study of the Canadian situation when skim milk was in surplus indicated that revenue losses incurred by subsidized SMP being sold in the export market could be cut by diverting SMP into the production of value-added casein and sodium caseinate (Canadian Dairy Commission, 1983), products presently not manufactured in Canada. Traditionally, acid casein has been produced by acidification, curd formation, washing and drying (Morr, 1985a). Sodium caseinate may then be produced by neutralization after washing the curd or using dried acid casein as the starting material. Although acid casein and caseinate production are well established technologies, Canada does not have processing facilities for the manufacture of these products and the capital investment and fluid milk throughput required is considered too great to consider developing such facilities.

Extrusion processing was conceived as a means of producing acid casein and sodium caseinate on demand directly from SMP as the starting material rather than fluid milk. As a continuous reactor based on a screw pump, an extruder conveys, mixes, and can subject a feed material to heat and/or pressure before being discharged through a die (Harper, 1981; Fichtali and van de Voort, 1989). Since our initial development trials for producing an acid casein (Barraquino et al., 1988), our research group has worked extensively on the development of a complete extrusion based process, first to produce an acid casein, followed by washing the curd and then a second extrusion step to neutralize the acid casein to produce sodium caseinate. Acid casein conversion to sodium caseinate using extrusion has been studied (Millauer et al. 1984; Boullé, 1986, 1987; Linko et al., 1986) and is used commercially. However, the concept of using SMP as a starting material and converting it to sodium caseinate is new. The production of caseinates from SMP differs substantially from the fluid milk process and required substantial investigation to characterize and optimize the physicochemical characteristics of the curd, yield, washing efficiency, and physicochemical and functional properties of the final product. Our objective was to determine the physicochemical and functional properties of sodium caseinate prepared from SMP by extrusion-processing relative to commercially available sodium caseinate.

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MATERIALS & METHODS

Processing

Coagulation of SMP. Commercial low-heat SMP (Crino Ltd. Agropur, Quebec) was used as the starting material and had the following proximate composition: 30.6% protein, 54% lactose, 0.8% fat, 7.8% ash, and 4.5% moisture. Coagulation of SMP was carried out with a Baker-Perkins MPF-50D (Baker Perkins F.E.S., Inc., Grand Rapids, MI) co-rotating intermeshing twin-screw extruder by pumping dilute reagent grade HCl into the barrel to mix with the incoming dry SMP. The coagulation process took place in the barrel by controlling the mixing and residence time to ensure the reaction was complete by the time the product exited from the die. No restrictive die insert was used in the acidulation process with the extruder barrel configured in a 15:1 L/D ratio with a screw profile designed to obtain a good balance between conveying and mixing action. Screw speed was set at 100 rpm with barrel temperature at 55°C resulting in a product temperature of 46.5± 1.0°C at screw speed 100 rpm. A complete description of acid casein production by extrusion has been reported by Fichtali and van de Voort (1990a). Two batches of acid casein were produced from skim milk powder, ESMP1 and ESMP2, both proceded at a feed rate of 15 kg/hr with acid delivered at a flow rate of 45 L/hr. The resulting acid casein output was ~25% solids and had pH's of 4.56 and 4.31 for ESMP1 AND ESMP2, respectively.

Washing and dewatering. The extruded coagulum was washed in a 100-L temperature-controlled tank fitted with a variable speed propeller. Four washing stages were used, each with a wash water ratio of 4:1 (water:coagulum), using water at the same pH as used for coagulation, stirring at 200 rpm, average residence time 20 min. The wash water temperature was 45°C for the first and last washes and 60°C for the second and third. For dewatering ESMP1, conventional screening (100 mesh) was used and a basket centrifuge (Model STM-1000, Western States Machine Co., Hamilton OH) was used for dewheying and dewatering ESMP2. The centrifuge was connected to the washing tank using a variable-speed positive displacement pump (Albin, Atlanta GA, Model 107SLP) and the centrifuge was run at 911 × *g* for the first 3 washing stages and 1615 × *g* for the last stage. Details concerning the washing procedure and its effects were reported by Fichtali and van de Voort (1990b).

Neutralization of acid casein. The dewatered curd (50–60% moisture) was ground and used as feed for the second extrusion step where it was mixed with NaOH at 2.5 to 2.8% by weight of casein solids entering the extruder, run at 95°C and 100 rpm to neutralize ESMP1 and ESMP2 to produce an end product with 70% moisture. In addition, dry commercial acid casein, obtained from Alacid, New Zealand, was also processed in a similar fashion. However, as it was a dry powder, the extruder was run at 150 rpm and a product with lower moisture content was produced (29%), subsequently referred to as extruded commercial acid casein (ECAC).

Spray drying. Due to atomizer limitations brought about by the high viscosities encountered, drying was carried out at 13% solids rather than the 20% normally used in industrial practice. The extruded sodium caseinate solution were maintained at 80°C in a 50-L agitated tank and pumped to a Niro spray dryer at 32 L/hr, operating with inlet

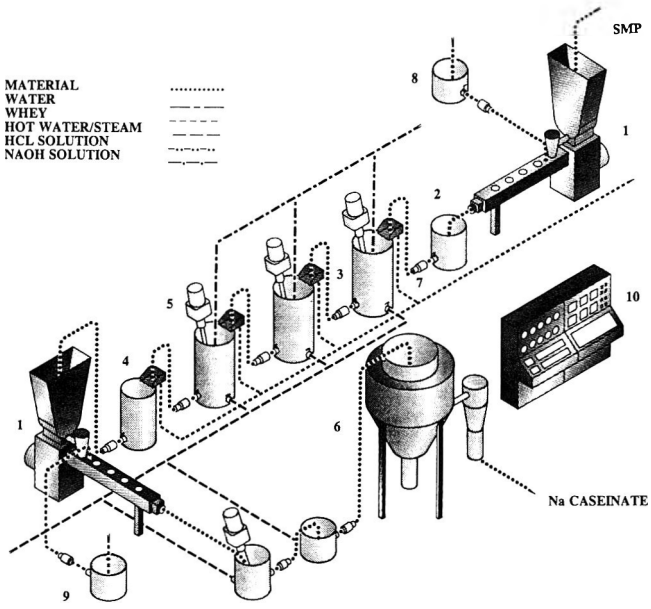

Fig. 1—Plant layout concept for caseinate production using extrusion technology. (1) extruder, (2) holding tank, (3) washing tank, (4) inclinded screen for separation, (5) agitator, (6) spray dryer, (7) pump, (8) supply tank for HCl solution, (9) supply tank for NaOH solution, (10) control panel.

195°C and 87°C. A schematic flow diagram of the complete process for the production of acid casein and sodium caseinate on an industrial scale is presented in Fig. 1.

Physicochemical analyses

Protein (micro-Kjeldahl), moisture, fat and ash were determined using standard AOAC (1984) procedures. Bulk density was determined by the method reported by Kinsella (1984) and sedimentable matter by the method described by Roeper (1977). Sample preparation for mineral analyses was carried out by wet digestion (AOAC, 1984) and mineral analysis performed using a Perkin Elmer 2380 atomic absorption spectrophotometer. Lactose analysis was carried out by the ISO (1980) method and pH measurements were made using an HI 8417 pH meter. Buffering capacity of proteins was measured using the method of Morr et al. (1973) and SDS-PAGE electrophoresis was performed according to the method of Weber and Osborn (1969).

Scanning electron microscopy (SEM) was carried out using a Cambridge Stereoscan 600 (Cambridge Instruments, England) at 15 kilovolts with the sample prepared by the procedure of Fleming et al. (1985). Color measurements were made using a Minolta Chroma Meter II reflectance colorimeter (Minolta Camera Co., Ltd., Osaka, Japan) using the CIE color measurement system. Commercial sodium caseinate (CSC) was used as control.

Functionality tests

Commercially available sodium caseinate (CSC) was used as control for all functionality tests. Water sorption isotherms at 25°C were constructed with the isopiestic method using H_2So_4 instead of saturated salt solutions. For isotherm measurements, the method of Ruegg and Moor (1984) was followed. However, the equilibrium moisture contents were based on dry weights obtained after storing samples for 2 wk over P_2O_5. The dry samples were weighed and placed in chambers with predetermined water activity (a_w) for 7 days at 25°C and then reweighed. Their a_w values were also measured using a Decagon water activity meter (Decagon Devices, Inc., Pullman, WA.) and the water content (%) plotted against a_w.

Protein solubility was measured by the method of Morr et al. (1985) and viscosity measurements were made with a Haake RV-20 Rotovisco equipped with an RC 20 Rheocontroller using a MV 1 rotor (Haake, Germany). Viscosity measurements were made using 10% protein solutions of each sample buffered at pH 5.0, 7.0 and 9.0 using two levels of ionic strength, 0.05 and 0.30μ. Shear stress vs shear rate curves were plotted over a 5 min period at a shear rate of 0 to 500 sec^{-1} at 23°C. Data were analyzed for their fit to Newtonian, Ostwald, Herschel-Bulkley, Casson, and other models and the best fit selected based on correlation coefficients, R^2.

Table 1—Physicochemical analyses of sodium caseinate samples[a]

	CSC	ECAC	ESMP1	ESMP2
Protein (%)	93.40	90.60	93.10	92.80
Moisture (%)	1.90	4.50	1.90	2.10
Lactose (%)	0.10	0.10	0.04	0.01
Ash (%)	3.60	3.70	3.90	4.00
Fat (%)	1.00	1.10	1.10	1.10
pH	6.85	6.50	6.85	6.72
Ca (ppm)	449	604	582	42
Cu (ppm)	1.35	6.4	3.69	4.10
Fe (ppm)	3.3	18.4	453	428
Mn (ppm)	0.25	3.79	2.02	1.12
Na (%)	1.34	1.25	1.36	1.66
Density (g/mL)	0.27	0.14	0.16	0.14

[a] Means of duplicates.

Foam expansion and foam stability were measured according to procedures reported by Morr (1985b). Emulsifying capacity was determined based on the turbidimetric method of Pearce and Kinsella (1978), with the absorbance of the diluted emulsions read at 500 nm (Yamauchi et al. 1982). Emulsion stability was also determined by the method of Yamauchi et al. (1982). The relative meltability of the caseinates was determined by microwave heating. 12% solutions (total wt, 70 g) were prepared at 40°C, adding 2.1 mL of 33% acetic acid with stirring, followed by 2.1 mL 33% sodium acetate. The precipitated curd was centrifuged, drained and 12g of curd was packed into a 12 mL circular mold. The formed sample was placed in the center of a 700-Watt domestic microwave oven (Sanyo, Tokyo, Japan) and heated at full power for 30 sec. The resulting melt area was measured with a Planix 5.6 digital planimeter (Tamaya and Co., Ltd., Osaka, Japan).

ANRC reference casein (Animal Nutrition Research Council, Teklad Test Diets, Madison, WI) was used as standard to determine the available lysine and relative nutritive value (RNV) of the samples. Available lysine was analyzed chemically by the Carpenter method (Carpenter, 1960) as modified by Booth (1971) and RNV by the procedure of Evancho et al. (1977) using *Tetrahymena furgasoni* ATCC 10542 (American Type Culture Collection, Rockville, MD). The microbiological quality of the samples was determined using colony counts obtained at 30°C (IDF, 1987), yeast and mold counts (IDF, 1985) and coliform counts (Hartman and LaGrange, 1985).

Statistical analyses

Color and functional property data such as meltability, available lysine and RNV were treated as completely randomized design experiments while solubility and foaming data were treated as randomized complete block design experiments (RCBD). Emulsification data were treated as a 4×2×3 factorial experiment in RCBD with 4 sodium caseinate samples, 2 ionic strengths and 3 pH levels as factors (Gomez and Gomez, 1984). The McGill University System for Interactive Computing (MUSIC) was used to carry out Analyses of Variance, Duncan's New Multiple Range Test of means and General Linear Model procedures of data (SAS Institute, Cary, NC).

RESULTS & DISCUSSION

Chemical properties

Protein, ash, Na, fat and pH varied only slightly among samples (Table 1). ESMP1 and ESMP2 contained substantially less lactose than CSC or ECAC. Bulk density of CSC was significantly higher than that of extruded products, and the calcium content of ESMP2 was approximately 10 times lower than its counterpart samples. The iron content in ESMP1 and ESMP2 was substantially higher than in CSC and ECAC. Electrophoresis indicated that all of the samples were similar in protein composition, with a major band corresponding to an average molecular weight (MW) of about 35,000. This was near the value reported by Basch et al. (1985) and within the range of relative molecular mass of 20 to 43 kD reported by Whitney et al. (1976). No whey protein bands were observed. However, a minor band at 63,000 Daltons was observed in CSC, ECAC, ESMP1, and ESMP2 and two minor bands (63,000

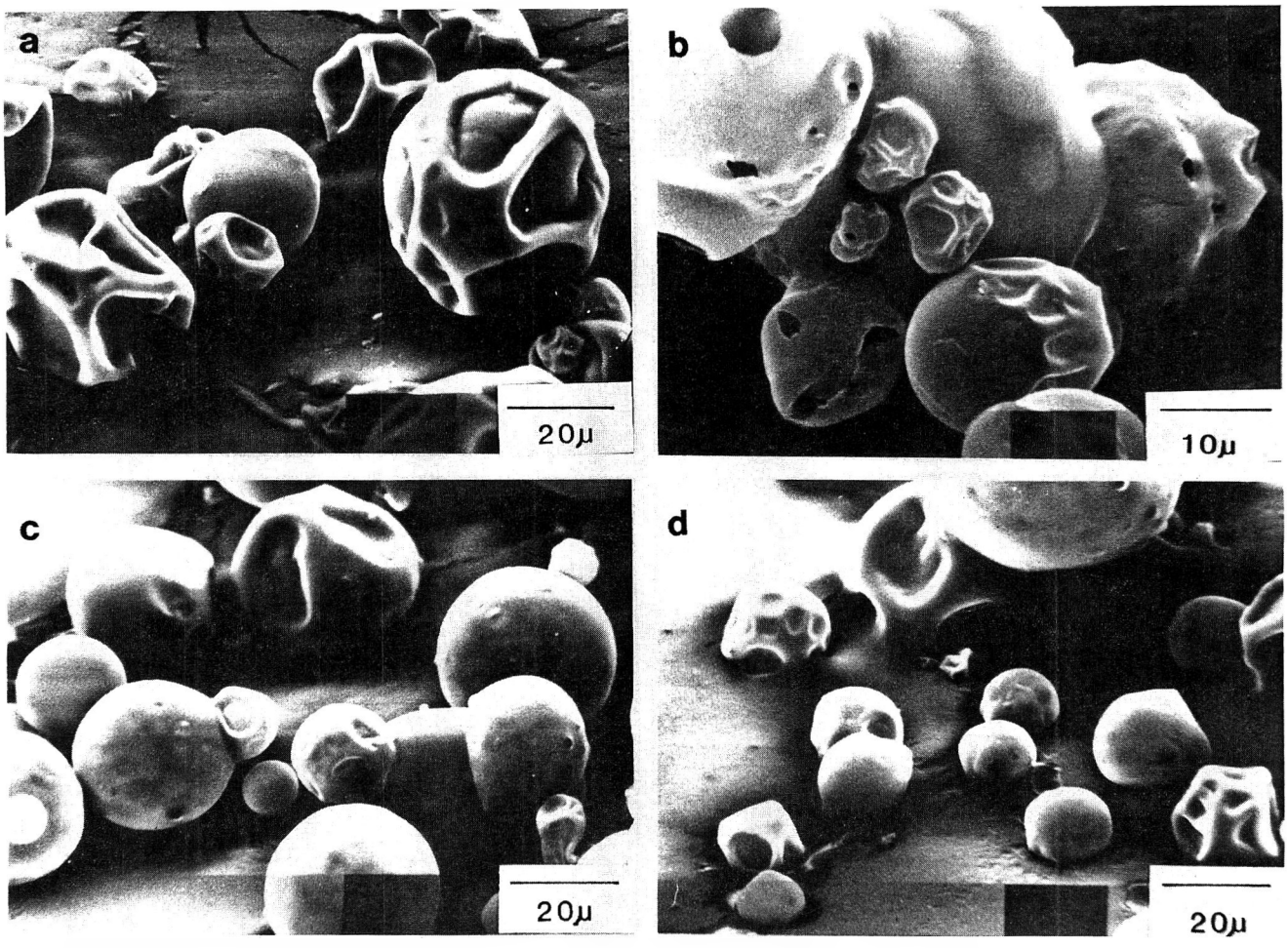

Fig. 2—Representative scanning electron micrographs of: (a) CSC, (b) ECAC, (c) ESMP1, and (d) ESMP2.

and 79,000 daltons) were present in ANRC reference casein. Those two bands have been suggested by Parnell-Clunies et al. (1988) to be disulfide-linked polymeric forms of kappa-casein. Minimum and maximum buffering capacities differed only slightly among samples. Minimum buffering capacity was observed to be at pH 4.4, 4.5, 4.5 and 4.9 for CSC, ESMP1 and ECAC, and ESMP2, respectively. ECAC, ESMP1, CSC and ESMP2 showed maximum buffering capacity at pH 5.8, 5.7, 5.6 and 5.6, respectively, similar to those obtained by Morr et al. (1973) and appeared to be characteristic of commercial caseinates.

Physical properties

The microstructures of the samples illustrated some differences between samples (Fig. 2a-d) with CSC tending to be indented spheres. ESMP1 and ESMP2 had fewer indentations but showed signs of agglomeration and surface pores. ECAC particles were also spherical but much more porous. The deep dents and folds in spray dried milk powders are due to the uneven shrinkage of casein during the drying process (Buma and Henstra, 1971), while agglomeration may be a function of the solubilization/moisture adjustment process prior to spray drying. In the case of ECAC, solubilization/moisture adjustment required about 4 hr resulting in more agglomeration and pore formation after spray drying.

With regard to color, all samples differed significantly from each other in terms of CIE chromaticity coordinates (x and y) and brightness factor Y (Table 2). These results compared very well with data of Southward (1978) for spray-dried sodium caseinate. Visually, however, only slight differences were detectable, CSC being the whitest product, followed by ESMP1

Table 2—Chromaticity values of sodium caseinate samples

	Y	x	y
CSC	87.25[a]	0.317[b]	0.326[a]
ECAC	82.00[d]	0.316[c]	0.324[b]
ESMP1	85.75[b]	0.313[d]	0.321[c]
ESMP2	82.80[c]	0.319[a]	0.326[a]

[a-d] Means of duplicates. Means with same superscripts not significantly different at $P < 0.05$.

which was quite similar. ECAC had a light grayish hue, while ESMP2, had a faint yellowish tint. Among all samples, ECAC had the highest sediment volume of 0.4 mL/10 mL, with the remaining samples ranging from 0 to <0.1 mL/10 mL. This indicated that both ESMP products were more efficiently converted to caseinate than ECAC. This was likely due to better mixing conditions fostered in the extruder facilitated by the higher moisture content (50-60%) of the ESMP feed relative to the dry commercial acid casein (Roeper, 1977).

Functional properties

The extruded samples, ESMP1 and ESMP2, adsorbed more water than CSC and ECAC, the ESMP samples showing a sharp increase in water uptake between a_w of 0.60 to 0.88. CSC and ECAC showed rapid water uptake at a_w 0.4 to 0.80 (Fig. 3). These differences may be related to microstructural and/or bulk density differences between samples. Differences in protein solubility among samples were apparent (Fig. 4) and pH had a highly significant effect on protein solubility ($P < 0.01$). CSC, ESMP1 and ESMP2 were more soluble than ECAC and all samples were more soluble at pH 7 than at pH 3. The inferior solubility of ECAC can be correlated with its higher

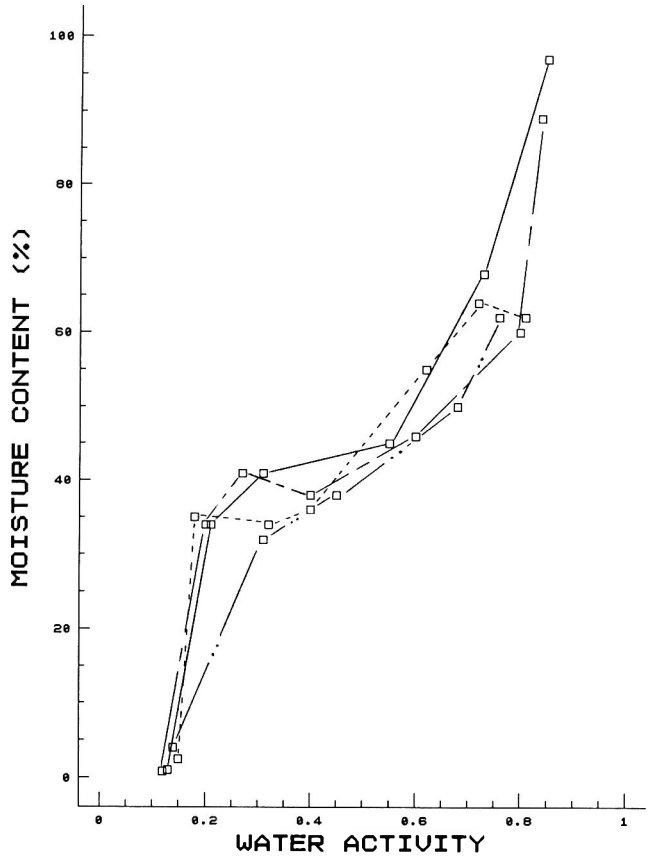

Fig. 3—Water vapor sorption isotherms of sodium caseinate samples: ECAC -----; CSC — ·· —; ESMP1 — - —; ESMP2. ———.

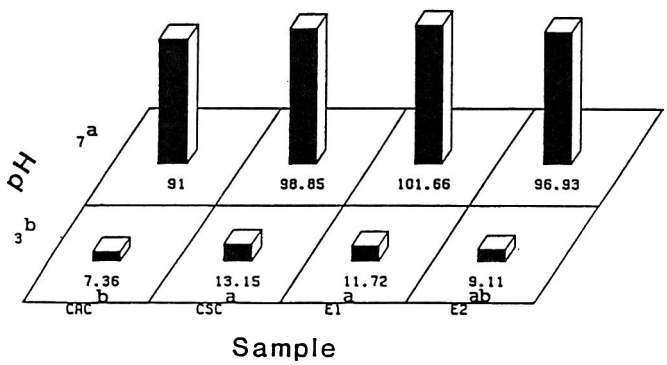

Fig. 4—Protein solubility of sodium caseinate samples (CAC=ECAC, CSC=CSC, E1=ESMP1 AND E2=ESMP2).

sedimentable matter content which indicated that it was not converted to caseinate as efficiently. Another factor which could have adversely affected its solubility characteristics was prolonged exposure to high temperature (4 hr at 80°C) during the solubilization/moisture adjustment process prior to spray drying.

Newtonian flow behavior characterized all samples, even with changes in pH or ionic strength, although viscosity itself was affected by these changes (Table 3), increasing with pH and ionic strength as reported previously by Hermansson (1975). The viscosity of samples differed to a slight extent, with ESMP2 having the highest viscosity followed by ECAC, ESMP1 and CSC, and could be related to sample solubility (Hermansson, 1975). The higher viscosity of ECAC vs CSC and ESMP1 may be attributable to higher calcium content (Hayes and Muller, 1961) and/or insoluble sedimentable matter (Canton and Mulvihill, 1982).

From the standpoint of foam expansion, no significant differences were observed (Table 4) among samples, however,

Table 3—Effect of ionic strength (μ) and pH on viscosity (Pa.s) of sodium caseinate samples[a]

	Viscosity					
	μ = 0.05			μ = 0.30		
Sample	pH 5	pH 7	pH 9	pH 5	pH 7	pH 9
CSC	0.02	0.03	0.03	0.01	0.03	0.06
ECAC	0.07	0.09	0.10	0.07	0.10	0.10
ESMP1	0.04	0.03	0.04	0.03	0.03	0.05
ESMP2	0.06	0.08	0.10	0.10	0.09	0.20

[a] Means of duplicates.

Table 4—Foam expansion and stability of sodium caseinate samples

	Expansion (%)	Stability (min)
CSC	81.54[a]	35.00[a]
ECAC	82.11[a]	21.00[b]
ESMP1	82.27[a]	23.83[b]
ESMP2	83.52[a]	24.00[b]

[a-b] Means of 6 determinations. Means with same superscripts not significantly different at P< 0.05.

Table 5—Emulsion turbidity (absorbance) and stability of sodium caseinate samples

	Absorbance (500 nm)	Stability (%)
CSC	0.158[b]	8.37[a]
ECAC	0.144[d]	5.33[b]
ESMP1	0.147[c]	5.93[b]
ESMP2	0.161[a]	6.33[b]

[a-c] Means of 12 determinations. Means with same superscripts not significantly different at P < 0.05.

differences in foam stability were highly significant (P < 0.01). CSC formed more stable foams than ESMP2, ESMP1 and ECAC. Both foam expansion and stability were significantly affected by pH (P < 0.01), with expansion and stability being better at pH 9 and 7 than at pH 4.5, where solubility was at a minimum. Emulsifying properties of the samples as measured by emulsion turbidity and stability were significantly different. Turbidity decreased in the order of ESMP2, CSC, ESMP1, and ECAC, while in terms of stability, CSC emulsions were superior to the other samples (Table 5). Again, this was likely a function of solubility (Yasumatsu et al., 1972), with higher concentrations of soluble protein facilitating formation of thicker interfacial films and smaller oil droplets, both more conducive to emulsion stability (Franzen and Kinsella, 1976). Ionic strength and pH affected both emulsion turbidity and stability significantly, with emulsions more turbid at μ = 0.05 but more stable at μ = 0.30. Increasing pH improved both turbidity and stability, likely due to an associated increase in viscosity (Morr, 1981). Melt area was measured as an indicator of cohesiveness of sodium caseinate as a function of temperature and is considered a useful indicator for applications such as mozzarella cheese production. The melt area of samples were significantly different from each other (P < 0.01) and correlated with solubility. CSC, which was the most soluble, had the largest melt area, 74.58 cm^2, while ECAC which was least soluble, had an area of 32.40 cm^2.

The available lysine content of ESMP1 and ESMP2 was significantly higher (P < 0.05, Table 6) than the ANRC reference casein. The CSC and ECAC results were in general agreement with published values (Carpenter and Bjarnason, 1969; Stahmann and Woldegiorgis, 1975; Holguin and Nakai, 1980). These data suggested that the extrusion conditions employed during acid coagulation coupled with the washing procedures to eliminate lactose, and extrusion to produce caseinate, were effective in conserving lysine. It was likely that the use of low-heat SMP as the starting material was also an important factor. Although the available lysine contents determined chemically were significantly different, the cell counts of *T. furgasoni* ATCC 10542 and their calculated RNV were not

Table 6—Available lysine, T. furgasoni *ATCC 10542 cell counts and relative nutritive value of sodium caseinate samples*

	Available lysine g/16g N	Counts cells/mL (10^3)	RNV (%)
CSC	7.9[b]	12.5[a]	83.3[a]
ECAC	6.7[b]	14.4[a]	95.8[a]
ESMP1	9.1[a]	13.8[a]	91.7[a]
ESMP2	9.1[a]	11.9[a]	79.2[a]
ANRC	7.4[b]	15.0[a]	100.0[a]

[a-b] Means of duplicates. Means with same superscripts not significantly different at $P < 0.05$.

(Table 6). This indicated that RNV was not solely a function of lysine availability, but other amino acids/nutrients contributed as well to nullify differences observed by the chemical method. The microbiologial counts of the samples were also assessed to provide an indication of overall microbiological quality. All samples were negative for coliforms and yeasts and molds. ECAC, ESMP1 and ESMP2 had mean total counts of $< 1 \times 10^3$ while CSC had the highest count of 12×10^3. The extrusion-based neutralization process, which exposed the feed material to temperatures of around 95°C was likely a key factor in obtaining such low counts.

The physicochemical and functionality tests only serve as a comparative guide for performance of extruded caseinates relative to commercial sodium caseinate. Severe limitations associated with functionality tests are well recognized in their subsequent translation to real food systems. From a physicochemical perspective all samples were comparable in terms of pH, protein, ash, Na, and fat contents, SDS-PAGE patterns, and buffering capacity. ESMP1 and ESMP2 contained substantially less lactose, had lower bulk density, and higher iron content than CSC. Lower lactose was obtained because of the extensive optimization applied in developing the washing and dewatering process. The lower bulk density was determined to be due to lower solids content used during spray drying and could be rectified (Neff and Morris, 1968; Tamsma *et al.*, 1969; Varshney and Ojha, 1975). The anomalous iron content in the extrusion process samples was likely due to leaching of iron from the barrel and screws caused by the acidic conditions (Cheftel, 1989). Microstructurally there were differences, although their significance is uncertain. CSC was superior in terms of color, however, the other samples were still very acceptable. From a functional standpoint, ESMP products were superior in terms of higher available lysine. All products were comparable in protein solubility, flow behavior, foam expansion, emulsion turbidity and relative nutritive value. CSC was superior to ESMP1 and ESMP2 and ECAC in terms of having lower water absorption characteristics, producing more stable foams and emulsions, and better melting properties.

CONCLUSIONS

ALTHOUGH DIFFERENCES were found between the products, overall, the sodium caseinates produced from SMP by extrusion appeared to be comparable to commercial sodium caseinate in general. Commerical acid casein converted to sodium caseinate by extrusion processing was generally judged inferior, however, the experimentally produced product may not be representative of industrial caseinate produced by extrusion. Data presented indicated that a good quality sodium caseinate with the requisite properties normally associated with commercial caseinate can be produced from SMP by extrusion processing. Use of such a process when SMP is in surplus could serve as a means of producing either acid casein or sodium caseinate on demand, involving substantially lower capital costs than traditional methods.

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Water Associated with Whey Protein Investigated by Pulsed NMR

GRACIELA W. PADUA, SHELLY J. RICHARDSON, and MARVIN P. STEINBERG

ABSTRACT

Proton longitudinal relaxation rates were determined for whey protein powders and suspensions of solids concentration between 0.01 and 2.0 g solids/g water. Data showed three hydration regions. The relationship between relaxation rate and solids content was investigated for each region. The two-state model with fast exchange was applied to the dilute suspensions and the high-solids regions. This allowed identification of polymer water and free water states in dilute suspensions and also identification of polymer water and capillary water in high-solids preparations. For intermediate-solids mixtures the two-state model was extended to three water states: polymer, capillary and free. These models enabled calculation of the amount of each water state associated with whey protein at a given moisture content.

Key Words: water-states, whey, protein, nuclear-magnetic-resonance, water, water activity, sorption isotherm, models

INTRODUCTION

WHEY, a by-product of the manufacture of cheese, contains about 20% of the total protein in milk. Whey protein is of a nutritional quality superior to casein. Aside from these facts, environmental concerns encourage development of useful applications for the large quantities of whey produced annually. Several techniques including ultrafiltration, gel filtration and ion exchange have been developed to recover protein from whey in the form of concentrates or isolates (Mulvihill and Fox, 1987).

In order to make more extensive use of skim milk components in the food industry, it is important to better understand the water relations of these materials. Many of their chemical and physical properties, as well as microbial stability, are greatly affected by the nature of water associations (Lang, 1980; Urbanski et al., 1982a,b). Lang (1980) applied the Smith sorption isotherm equation (Smith, 1947) to carbohydrate and protein sorption data and defined two states of water associated with those macromolecules. The water sorbed in the water activity (a_w) range of 0.3 to about 0.9 was termed polymer water (POL) and that sorbed above this range was termed capillary water (CAP). These two water states were also detected by low-field pulsed proton NMR spectroscopy for casein and starch.

Pulsed NMR techniques, both low-field and high-field, have been successfully applied to the study of water relations in biological materials (Bryant and Shirley, 1980; Nagashima and Suzuki, 1981). All three possible nuclei, ^1H, ^2H, and ^{17}O, have been probed and various models for data interpretation have been developed (Halle et al., 1981; Kumosinski and Pessen, 1982; Derbyshire, 1982; Lioutas, 1984; Pessen et al., 1985). An extensive review of the subject may be found in Richardson and Steinberg (1987). Most of the data were collected probing the ^1H nucleus because of its high sensitivity, although problems in data intepretation due to chemical exchange (Berliner and Reuben, 1980) and cross-relaxation (Edzes and Samulski, 1978) have been discussed. Pessen et al. (1985) concluded that, although cross-relaxation contributes to the longitudinal relaxation process, especially at high frequencies, data are useful to monitor changes or trends in the hydration of the protein. Chinachoti and Steinberg (1989) employed low-field proton NMR to determine the amount of POL and SOL (solute) water in starch-sucrose slurries. Their determinations showed a high correlation with POL and SOL calculated from sorption isotherm data for the same system.

Zimmerman and Brittin (1957) provided theoretical grounds to study the distribution of water molecules into different states. When the exchange of nuclei among these states is very rapid compared to relaxation times

$$\frac{1}{T_{obs}} = \sum_{i=1}^{i=n} \frac{P_i}{T_i} \qquad [1]$$

where T_{obs} is the observed relaxation time T_1 or T_2, T_i is the relaxation time of the i th water state, and P_i is the probability of the nucleus being in that state. For dilute polymer suspensions two water states were considered, bound (B) and free (F). Equation (1) then becomes the "two state model with fast exchange."

$$\frac{1}{T_{obs}} = \frac{P_B}{T_B} + \frac{P_F}{T_F} \qquad [2]$$

Since P_F equals (1 - P_B), and P_B can be related to the solids concentration of the suspension, Equation (2) predicts a linear behavior of the relaxation rate ($1/T_{obs}$) with polymer concentration in the suspension. This behavior has been observed experimentally for protein and starch suspensions of low solids concentration. However, deviations occur at higher solids content (Lelievre and Mitchell, 1975; Lioutas et al., 1986; Richardson et al., 1987), presumably as a result of macromolecules packing closer together forming capillaries where water molecules are immobilized to some extent (Richardson et al., 1985; Roefs et al., 1989). Roefs et al. (1989) concluded from their proton NMR study on casein dispersions that interpretation of the relaxation time exclusively in terms of bound and free water was not possible, and that a more general approach was needed to include the water captured in cavities and between macromolecules. The objective of our study was to investigate the states of water associated with whey protein by applying Zimmerman and Brittin's theory to low-field pulsed proton NMR measurements on whey protein-water mixtures.

MATERIALS & METHODS

THE WHEY PROTEIN was Bi-Pro Brand (Le Sueur Isolates, Le Sueur, MN) which had been prepared by isolating it from cheese whey by ion exchange chromatography. The pure protein suspension was concentrated by ultrafiltration and spray-dried. The isolate contained 95% undenatured whey protein, 3% minerals, 1% fat, and 1% lactose (on a dry basis). A sorption isotherm was determined for the whey protein by equilibration against salt slushes in proximity equilibration cells (Lang et al. 1981). Results were plotted using the Smith sorption

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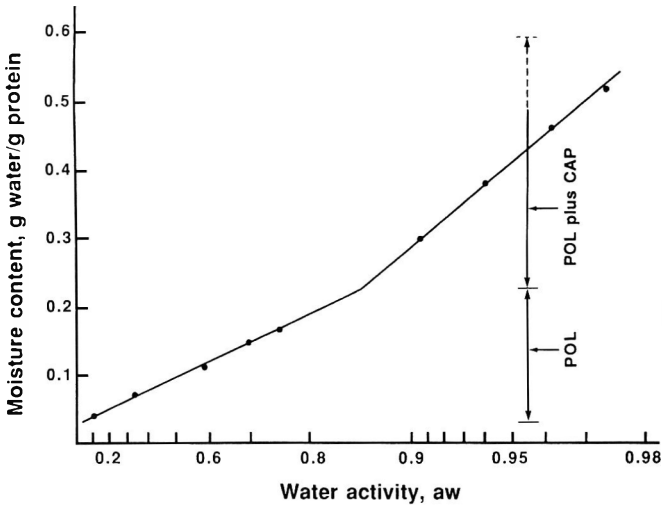

Fig. 1.—Sorption isotherm for whey protein.

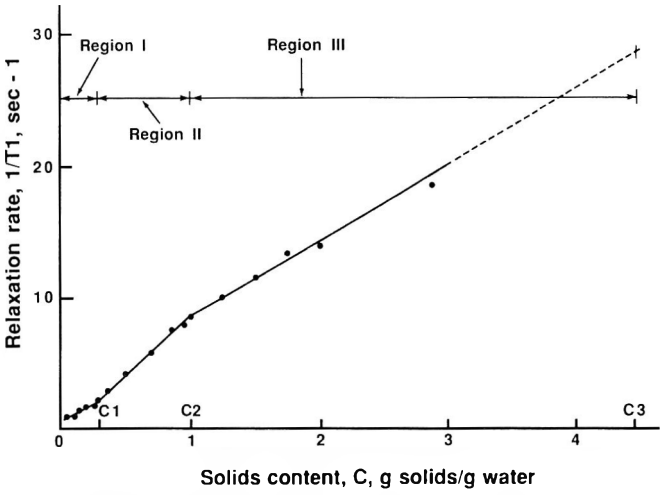

Fig. 2.—Variation of proton relaxation rate of whey protein suspensions with solids content.

isotherm (Smith, 1947) according to Lang and Steinberg (1981) to obtain a linear isotherm.

The proton longitudinal relaxation time (T_1) of whey protein suspensions and high-moisture powders was determined at 20°C with a 10 MHz Pulsed NMR spectrometer (PR-103, Praxis Corp, San Antonio, TX) with a 90°-τ-90° pulsed sequence. Solids content of samples covered the range from 0.01 to 2.9g solids/g water. Samples with low solids content up to 1 g solids/g water were prepared by direct addition of water to the protein. Samples with higher solids content were hydrated by exposing the protein to a salt slush at 0.97 a_w or to water in proximity equilibration cells (Lang et al., 1981) for limited periods of time. Relaxation was exponential at every solids content. T_1 was calculated by a microprocessor connected to the spectrometer. Typically, ten points of the curve log amplitude vs. time were used in this calculation. The correlation factors were of the order 0.996. Values reported are averages of four determinations with an estimated error of 3%. In addition, proton T_1 measurements were carried out in the presence of D_2O in order to detect cross-relaxation effects (Myers-Betts and Baianu, 1990). These measurements gave no indication of any notable contribution of cross-relaxation to the observed relaxation rate at 10 MHz.

RESULTS & DISCUSSION

Data

Figure 1 shows the Smith sorption isotherm for whey protein. Two linear segments were observed; the line from a_w 0.11–0.85 corresponded to POL and the one above 0.85 a_w corresponded to POL plus CAP (Lang, 1980). The intersection of the two lines occurred at 0.225 g water/g solids (4.44 g solids/g water). These sorption data were in good agreement with published reports (Greig, 1979).

Figure 2 shows the solids content dependence of longitudinal relaxation rate ($1/T_1$). Three linear regions were observed. The first region ended at $C_1 = 0.28$ g solids/g water, the second ended at $C_2 = 1$g solids/g water, and the third region extended from C_2 beyond the experimental range to $C_3 = 4.45$g solids/g water. The value of C_3 was the moisture content at the intersection of the lines representing POL and POL + CAP in the sorption isotherm (Fig. 1). C_3 was the lowest solids content at which CAP began to appear.

Similar graphs of the dependence of oxygen-17 and deuterium relaxation rate on concentration were reported by Richardson et al., (1987) for corn starch suspension. They presented a physical model to explain their observations. Relaxation rate behavior at low solids content (10–40%) was consistent with the two-state model with fast exchange. The increased gardient of the intermediate concentrations (40–60%) was considered due to a change in the relaxation rate of bound water. The second change in gradient for high solids preparations (60–70%) was attributed to the presence of trapped water between starch granules. The same report presented a second chemical model to explain the data. In that model, gradient changes were ascribed to interactions between starch granules and described in terms of starch "activity" in the suspensions. That treatment illustrated the fact that relaxation measurements for the solvent were useful to gain insight on the behavior of the solute. Data presented in Fig. 2 were treated according to the physical model presented by Richardson et al., (1987).

Two-state model with fast exchange

The linear dependence of the relaxation rate on solids content for dilute solutions, shown in Region I of Fig. 2, was predicted by the two-state model with fast exchange (Eq. 2). In that case, the fast exchange model was adopted in view of the exponential nature of the relaxation at every protein concentration (Gadian, 1982). The bound water was identified as POL, and the free water as FRE. If the probability of the water being in each state, P_B and P_F, is replaced by the fraction (f) of the water in that state (Lelievre and Mitchell, 1975), equation 2 becomes

$$\frac{1}{T_{obs}} = \frac{f_{POL}}{T_{POL}} + \frac{f_{FRE}}{T_{FRE}} \qquad [3]$$

Since f_{FRE} equals $(1 - f_{POL})$, Eq. (3) gives

$$\frac{1}{T_{obs}} = \frac{f_{POL}}{T_{POL}} + \frac{1 - f_{POL}}{T_{FRE}} = f_{POL}\left[\frac{1}{T_{POL}} - \frac{1}{T_{FRE}}\right] + \frac{1}{T_{FRE}} \qquad [4]$$

which predicts a linear behavior of $1/T_{obs}$ with f_{POL}. Blanshard and Derbyshire (1975) reported a linear dependence of the longitudinal relaxation rate on concentration for a myosin solution. The gradient of this line was expressed as $h(1/T_{bound} - 1/T_{fre})$, where h was the hydration number in g bound water/g macromolecule. They pointed out that knowledge of the gradient alone was insufficient for the determination of the hydration parameters and the relaxation rate of the bound phase. To obtain those values, it was necessary to use another quantity. The procedure to estimate hydration values used by Blanshard and Derbyshire (1975) was NMR signal attenuation on freezing. This procedure was not considered completely satisfactory because often no unique value was obtained. In our work, the sorption isotherm in Fig. 1 was used to relate f_{POL} to the solids concentration (C); the break in the curve, which occurred at 0.225 g water/g protein, was taken as a measure of the maximum value of POL; therefore

$$f_{POL} = C \frac{\text{g solid}}{\text{g water}} \times 0.225 \frac{\text{g POL}}{\text{g solids}} \qquad [5]$$

Substituting this expression for f_{POL} in Eq. (4) results in a linear relationship of $1/T_{obs}$ with solids content.

Region III of Fig. 2 corresponds to the upper line of the sorption isotherm (Fig. 1) where water is present in two states, POL and CAP. In that case, the two-state model was applied to POL and CAP; Eq. (2) becomes

$$\frac{1}{T_{obs}} = \frac{f_{POL}}{T_{POL}} + \frac{f_{CAP}}{T_{CAP}} = \frac{f_{POL}}{T_{POL}} + \frac{1 - f_{POL}}{T_{CAP}} \quad [6]$$

$$= f_{POL}\left[\frac{1}{T_{POL}} - \frac{1}{T_{CAP}}\right] + \frac{1}{T_{CAP}} \quad [7]$$

When f_{POL} is replaced by Eq. (5) a linear relation for $1/T_{obs}$ with solids content is obtained.

Development of the extended model

Region II (Fig. 2) lies between Region I for POL and FRE and Region III for POL and CAP. This line had a negative intercept. However, Eq. (7), which was derived on the basis of a two-state system, had a positive intercept. Therefore, Region II cannot be represented as a two-state system. To explain this behavior, the coexistance of the three water states, POL, CAP and FRE, is proposed. This hypothesis can be expressed as:

$$\frac{1}{T_{obs}} = \sum_{i=1}^{i=3} \frac{f_i}{T_i} = \frac{f_{POL}}{T_{POL}} + \frac{f_{CAP}}{T_{CAP}} + \frac{f_{FRE}}{T_{FRE}} \quad [8]$$

If f_{FRE} is expressed as $(1 - f_{POL} - f_{CAP})$ Eq. (8) becomes

$$\frac{1}{T_{obs}} = \frac{f_{POL}}{T_{POL}} + \frac{f_{CAP}}{T_{CAP}} + \frac{1 - f_{POL} - f_{CAP}}{T_{FRE}} \quad [9]$$

Since Region I does not contain CAP, $f_{POL} = 0$ at C_1. Region III does not contain FRE; therefore, at C_2, $f_{FRE} = 0$ and $f_{CAP} = 1 - f_{POL}$. To simplify Eq. (9), CAP in Region II was assumed to increase linearly with solids content and f_{CAP} was expressed in terms of f_{POL}:

$$f_{CAP} = f_{POL}\left[\frac{1 - f_2}{f_2 - f_1}\right] - f_1\left[\frac{1 - f_2}{f_2 - f_1}\right] \quad [10]$$

Where f_1 and f_2 are the values of f_{POL} at C_1 and C_2, respectively. Assigning a to the slope and b to the intercept of Eq. (1), and substituting Eq. (10) in Eq. (9), gives

$$\frac{1}{T_{obs}} = \frac{f_{POL}}{T_{POL}} + \frac{af_{POL} - b}{T_{CAP}} + \frac{1 - f_{POL} - (af_{POL}) - b)}{T_{FRE}}$$

$$= f_{POL}\left[\frac{1}{T_{POL}} + \frac{a}{T_{CAP}} - \frac{a+1}{T_{FRE}}\right] - \left[\frac{b}{T_{CAP}} - \frac{1+b}{T_{FRE}}\right] \quad [11]$$

when Eq. (5) is substituted in Eq. (11), the result is a linear relationship of $1/T_{obs}$ with solids content as found in Region II in Fig. 2.

Determination of model constants

In order to evaluate the models presented, it was necessary to determine the constants T_{FRE}, T_{POL}, and T_{CAP} for whey protein. The value of T_{FRE} should correspond to T_{obs} of pure water. This value could not be determined with the instrument used, because pure water showed a very high signal intensity that saturated the instrument receiver; so it did not give a repeatable signal. Therefore, T_{FRE} was estimated by extrapolating the Region I line in Fig. 2 to C = 0. The value found $1/T_1 = 0.4$ sec^{-1}, was in excellent agreement with reported values (Glasel, 1972). This fact verified the quality of data for Region I and gave an independent estimation of T_{FRE}. The value of T_{POL} corresponded to T_{obs} at C_3 of Fig. 2, the end of the capillary region. This value could not be determined with the instrument used. However, $1/T_{POL}$ could be estimated by extrapolating the Region III line in Fig. 2 to C_3. The value found was $1/T_1 = 27.86$ sec^{-1}. The value of T_{CAP} was estimated from Eq. (7), at $f_{POL} = 0$, Eq. (7) becomes $1/T_{obs} = 1/T_{CAP}$. The value found by extrapolating the line for Region III to C = 0 in Fig. 2 was $1/T_1 = 3.34$ sec^{-1}. Thus, the constants in the Region I and Region II models were evaluated independently of experimental data. This allowed a valid comparison of those models with the linear regression of the experimental points.

Table 1—Proton relaxation rates of whey protein suspensions at different concentration levels

Region	g solid/g water	$1/T_1$ observed sec^{-1}	$1/T_1$ calculated sec^{-1}	Deviation %
I	0.1	1.02	1.02	0.4
	0.2	1.61	1.63	1.1
II	0.5	4.14	4.20	1.4
	0.8	6.80	7.00	2.9

Region models

The hypothesis presented was summarized in the equations given below which related $1/T_{obs}$ to C for each of the linear regions (Fig. 2):

Region I (Eq. 4): $1/T_{obs} = 6.17\,C + 0.4$ where $0 < C < 0.28$.
Region II (Eq. 7): $1/T_{obs} = 9.34\,C - 0.46$ where $0.28 < C < 1$
Region III (Eq. 11): $1/T_{obs} = 5.51\,C + 3.34$ where $1 < C < 4.45$

Relaxation data in Region III were collected up to 2.9g solids/g water, the limit of the instrument sensitivity range; however, sorption isotherm data showed this region to continue up to 4.45g solids/g water. The appropriate model equation was used to calculate $1/T_1$ for two levels of solids concentration (C) in regions I and II. These calculations are compared with the observed (from linear regression) values in Table 1. The observed and the calculated values of $1/T_1$ were in excellent agreement as shown by the low deviations.

This close agreement between observed and calculated relaxation rates, and the fact that the constants T_{POL} and T_{FRE} were obtained independently of experimental points for Region I, validates the two-state POL and FRE, model for Region I. That is, provided the assumptions of nonsignificant cross-relaxation effect and fast exchange between protons are correct. Since evaluation of the constant T_{POL}, T_{CAP} and T_{FRE}, which appear in the model for Region II, was independent of the experimental points in this region, the three-state model was considered valid for Region II (provided assumptions made for the Region I model are correct). The constants for Region III, T_{POL} and T_{CAP}, were obtained from Region III data, i.e., not from an independent measurements. However, these constants had been validated when the Region II model was tested. Also, the two-state model, POL and CAP for Region III is supported by the sorption isotherm data of Fig. 1 and it is consistent with published reports (Glasel, 1972). Thus, the Region III model was considered valid.

Calculation of the amount of water states

Since the models presented in Eq. (4), and (9) fitted the corresponding hydration regions, the expressions for f_{POL} in Eq. (5) and f_{CAP} in Eq. (10) were used to calculate the amount of each water state associated with whey protein at a given moisture content. To illustrate this point three levels of moisture content, one for each region, were arbitrarily chosen and the calculated amounts of the different water states are shown in Table 2. In Region I two water states were present, polymer and free water. The fraction of total water present as polymer water was calculated from Eq. (5). Thus, at the concentration

Table 2—Amount of water states in whey protein suspensions of different concentration levels

g solid/g water	Hydration region	Water states POL	(% of total water) CAP	FRE
0.15	I	3.4		96.6
0.75	II	16.98	50.3	32.8
1.50	III	33.8	66.2	

of 0.15 g solids/g water, in Region I, the amount of polymer water was

$$f_{POL} = 0.15 \frac{\text{g solids}}{\text{g total water}} \times 0.225 \frac{\text{g POL}}{\text{g solids}} = 0.034 \frac{\text{g POL}}{\text{g total water}}$$

This allowed the amount of free water to be calculated from Eq. (4):

$$f_{FRE} = 1 - 0.034 = 0.966 \frac{\text{g FRE}}{\text{g total water}}$$

The water states present in Region II were polymer, capillary and free. The amount of polymer water was calculated again by use of Eq. (5). Thus, at the concentration of 0.75 g solids/g water, in Region II, the amount of polymer water was

$$f_{POL} = 0.75 \frac{\text{g solids}}{\text{g total water}} \times 0.225 \frac{\text{g POL}}{\text{g solids}} = 0.169 \frac{\text{g POL}}{\text{g total water}}$$

Capillary water was obtained from Eq. (10). To use this equation it was necessary to calculate first the two values for f_{POL}, f_1 and f_2 from Eq. (5). At $C_1 = 0.21$ g solids/g water (taken from Fig. 1), $f_1 = 0.065$ and at $C_2 = 1.0$ (from Fig. 1), $f_2 = 0.225$ g POL/g total water. The amount of capillary water at the concentration of 0.75 g solids/g water was

$$f_{CAP} = 0.169 \times \frac{1 - 0.225}{0.225 - 0.065} - 0.065 \times \frac{1 - 0.225}{0.225 - 0.065} = 0.503 \frac{\text{g CAP}}{\text{g total water}}$$

The amount of free water was given by Eq. (9):

$$f_{FRE} = 1 - 0.169 - 0.503 = 0.328 \frac{\text{g FRE}}{\text{g total water}}$$

In Region III, the water states present were polymer and capillary water. The amount of polymer water was again obtained from Eq. (5). Thus, for the concentration of 1.5 g solids/g water, which was in Region III, the amount of polymer water was $f_{POL} = 1.5 \times 0.225 = 0.338$ g POL/g total water. The amount of capillary water was given by Eq. (7). Thus, at the same concentration $f_{CAP} = 1–0.338 = 0.662$ g CAP/g total water.

Activity model

The dependence of relaxation rate on concentration for wheat flour (Richardson et al., 1986), β-lactoglobulin (Kumosinski and Pessen, 1982), and corn zein (Myers-Betts and Baianu, 1990) preparations has been fitted with the exponential model:

$$R_{obsd} = n_H R_B c e^{2B_0 c + 1.5 B_2 c^2} + R_F$$

where R_{obsd} is the observed relaxation rate (sec^{-1}), n_H is the hydration value (g water/g solids), c is the concentration (g solids/g water), R_B is the relaxation rate for the bound water (sec^{-1}), R_F is the relaxation rate for free water (sec^{-1}), B_0 and B_2 are the coefficients of the virial expansion of osmotic pressure in terms of concentration. This model resulted from use of activities (Kumosinski and Pesser., 1982) instead of concentrations in the two-state model with fast exchange (Zimmerman and Brittin, 1957), and by expressing the activity coefficient in the form of a virial expansion of the sample concentration.

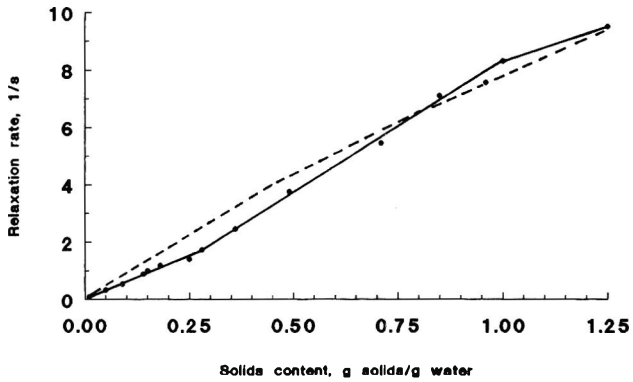

Fig. 3.—Relation between proton relaxation rate of whey protein suspension and solid content; exponential (----) and 3-line model fittings.

Data in Fig. 2 were fitted with the exponential model using the non-linear regression procedure (Marquardt method) of the SAS package (SAS Institute, Inc., 1985) on an IBM mainframe. The estimated parameter values and their 95% confidence interval were 8.25 ± 1.03 for $n_H R_B$, -0.03 ± 0.06 for B_0, and -0.003 ± 0.019 for B_2. The exponential model using these coefficients was plotted in Fig. 3 as a dashed line. This graph shows that the three linear segments model gave a better fit of the experimental points than did the exponential model.

CONCLUSIONS

THE DATA presented indicate that low-field pulsed proton NMR is a useful technique to follow changes in the hydration of whey protein. Three hydration regions were detected over the range of solids content covered. The application of the two-state model with fast exchange and of the extended model to three states, allowed characterization of 3 different water states associated with whey proteins. Two water states, polymer and free, were characterized in dilute suspensions by relaxation rates of 28 and 0.4 sec^{-1}, respectively. Three water states, polymer, capillary and free, were identified for the intermediate solids range and 2 states, polymer and capillary, were characterized in the high-solids region. Capillary water relaxation rate was 2.75 sec^{-1}. The models developed for each hydration region and use of the sorption isotherm enabled quantification of the amount of each water state present at a given total moisture content. Polymer water was calculated from the moisture sorption isotherm, capillary water was obtained from the model corresponding to Region II and free water was, in each case, the remainder of the total water that was not accounted for as polymer or capillary states.

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Incidence and Heat Resistance of *Clostridium botulinum* Type E Spores in Menhaden Surimi

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ABSTRACT

Raw menhaden surimi (*Brevoortia tyrannus*) was examined to determine the incidence of *Clostridium botulinum* spores. Seven of 565 test portions (1.2%) were positive for type E spores. The thermal death time (TDT) tube method was used to determine the heat resistance of *C. botulinum* type E spores inoculated into the remaining 558 negative portions. Calculated mean D-values in mm were 8.66 at 73.9°C, 3.49 at 76.7°C, 2.15 at 79.4°C, and 1.22 at 82.2°C. The z-value of the phantom TDT curve was 9.78C°. Our data indicate previously reported minimal time/temperature thermal processes used to set surimi gel provide an adequate margin of safety with regard to *C. botulinum* type E.

INTRODUCTION

SURIMI, a food product of minced and washed fish flesh, because of high protein, low-fat content, possesses unique gel-forming capabilities upon heating (Lee, 1984). These enable its use in formulated seafood analogs such as imitation crab, lobster, scallop and shrimp. Surimi and its related products were generally unavailable to U.S. consumers until the late 1970s, when surimi crab-leg analogs were introduced as a result of a severe decline in the Alaskan king crab industry. At that time, most surimi was prepared from Alaskan pollock in Japanese shore-based and/or factory ships. Production and consumption of surimi-based foods in the U.S. are projected to increase during the 1990s.

As the surimi market expands, considerable efforts are being made to identify species of fish that are suitable for use in surimi and to develop such under-utilized species as Atlantic and Gulf Coast menhaden (*Brevoortia tyrannus* and *Brevoortia patronus*, respectively) for that purpose. Menhaden are abundant, representing over 40% of the total U.S. fish landings (Cross, 1986). They are used for fish meal and oil, but are generally not accepted as a food source because of high fat content, fine bone structure and pronounced flavor and odor (Johnson et al., 1988). Menhaden are often harvested close to shore, and because the pathogen *Clostridium botulinum* may also be found in those same environments (Smith and Sugiyama, 1988), the possible occurrence of *C. botulinum* spores in menhaden flesh is of concern. Our preliminary research showed that menhaden surimi without cryoprotectants and inoculated with *C. botulinum* type E spores supported growth and toxin production by *C. botulinum*. Our current study, therefore, was undertaken to determine both the incidence and heat resistance of *C. botulinum* type E spores found in raw menhaden surimi.

MATERIALS & METHODS

Collection of surimi

A total of 565 raw menhaden surimi specimens, without added cryoprotectants, were obtained from the Zapata Haynie Corp. (Reedsville, VA). Portions weighing 454 to 907g each were shipped frozen to the Food & Drug Administration (FDA) laboratories during Atlantic coast harvest seasons and kept frozen until used.

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Incidence study

Experimental procedure. In a laminar flow hood, 250-g portions of raw menhaden surimi were aseptically placed in 1-L bottles containing 500 mL of sterile, freshly steamed and cooled trypticase-peptone-glucose-yeast extract (TPGY) broth and incubated at 26°C for 7 days. One portion in each test was prepared in duplicate and inoculated with about 10 spores of *C. botulinum* type E to serve as a positive control. After incubation, the cultures were centrifuged at 7000 × *g* for 30 min. The clear supernatant fluid was trypsinized, diluted 1:10 in gel-phosphate buffer and tested for C. *botulinum* toxin by the standard mouse bioassay (FDA *Bacteriological Analytical Manual*, 1984). Toxic cultures were typed by toxin neutralization tests when necessary.

Heat-resistance study

Preparation of sterile surimi. About 25 menhaden surimi samples, each weighing about 907g and found negative in the incidence study, were pooled and thoroughly mixed to obtain a homogeneous product; 454-g portions were then frozen and held at −20°C until needed. These portions were thawed under refrigeration and autoclaved for 60 min at 121°C to ensure destruction of any naturally occurring heat-resistant spores—a problem previously reported by Lynt et al. (1977).

Strains and spore stocks

A pooled spore mixture consisting of five strains of *C. botulinum* type E was prepared. Four of the strains (Beluga, Birmingham Salmon, 070 and G21-5) were obtained from the FDA spore stock. The fifth, MS-272, was isolated from a positive specimen obtained from the incidence study. Spore stocks were prepared from each strain as described by Lynt et al. (1983) and stored in sterile distilled water. Each spore suspension was diluted with sterile distilled water, and equal numbers of spores from each strain were mixed to form a single pooled spore mixture containing 1×10^7 spores/mL. The mixture was stored at 4°C and used throughout the study. Spores were enumerated by the 3-tube most probable number (MPN) method, with TPGY broth as the culture medium.

Inoculation of sterile surimi. Sterile surimi was ground in a sterile meat grinder and inoculated to obtain a concentration of about 1×10^5 C. *botulinum* spores/g. The seeded surimi was ground twice more to ensure homogeneity of the spore stock, tubed and stored in an ice bath for the duration of the experiment. At least five representative tubes, each containing 1g of surimi, were later used to determine the spore count by the 3-tube MPN method.

Experimental procedure. Thermal death time (TDT) experiments were conducted by the TDT tube technique at 73.9, 76.7, 79.4, and 82.2°C. The inoculated surimi was loaded into 10 × 75 mm heat-resistant glass TDT tubes in 1-g portions; the tubes were flame-sealed and stored in an ice bath until needed. Specimens were removed from the ice bath only long enough to be bundled into groups of 3 or 4 and attached to a sinker and identifying tag. Enough tubes were prepared for each experiment so that 100 tubes were dropped in groups of 10 into a precisely temperature-controlled silicon oil bath at selected time intervals. All tubes were removed simultaneously at the end of the experiment and immediately returned to the ice bath. Precision of oil bath temperature was indicated by a straight line recording from monitoring thermocouples. Those used for this purpose had been previ-

Table 1—Mean calculated D-values for Clostridium botulinum *type E spores in menhaden surimi.*

°C	Mean D-value[a] (min)	Range[b]
73.9	8.66 ± 0.76	6.80 – 12.97
76.7	3.49 ± 0.16	2.38 – 4.07
79.4	2.15 ± 0.21	1.10 – 1.65
82.2	1.22 ± 0.15	0.49 – 0.74

[a] D-value ± one standard deviation.
[b] Lynt et al., 1982.

ously checked against a calibrated thermometer over the temperature range used. Accuracy of the thermocouples was checked periodically.

Each experiment covered several time intervals, ranging from one that permitted survivors in all 10 tubes, to one in which no survivors remained in any of the tubes. A gradation between those extremes was developed so that some tubes within all but the final intervals would contain surviving spores. Four replicate experiments were performed at each temperature except 82.2, where we used three replicates.

The tubes were returned to the ice bath and held there until they were opened for culturing. Contents of the tubes were cultured in separate tubes of TPGY broth, each containing an inverted fermentation tube. This was necessary because the turbidity of the medium after surimi had been added obscured visible growth, and production of gas might be missed. All cultures were incubated for 2 wk at 26°C or until they showed evidence of growth and gas. All remaining negative tubes were then sealed with sterile Vaspar and further incubated at 26°C for at least 6 mo to allow heat-damaged spores an opportunity to recover, germinate and grow (Lynt et al., 1983). All cultures from the longest time interval that showed growth in all 10 tubes and any culture from longer times that showed growth were tested for botulinal toxin. Because contamination was possible during preparation of the surimi and in filling and opening tubes, only cultures found to contain toxin were counted among those with spores that survived heat treatment. Toxicity testing and typing were by standard mouse bioassay, as described.

Calculations and statistical analysis

Decimal reduction times (D-value) were calculated by using the Spearman-Karber estimation of the LD50 (Pflug and Holcomb, 1983). This indicates the time required to produce sterility in 50% of the tubes (corresponding to 0.69 surviving spores per tube). The D-value equals LD50/[log 10 (initial spore concentration) + 0.2507]. Exposure times were corrected for thermal lag and lethality during lag heating and cooling (Stumbo, 1965). The z-value was estimated by computing the linear regression (Ostle and Mensing, 1975) of log 10 (D-value) versus temperature (C). An estimate of z was obtained by computing the absolute value of the inverse slope.

RESULTS & DISCUSSION

SEVEN of the 565 test portions of menhaden surimi without added cryoprotectants examined, each weighing 250g, contained *C. botulinum* type E spores. The relatively low overall incidence (1.2%) was expected in view of the multiple washing and pressing procedures used in the production of surimi. The use of raw menhaden surimi without added cryoprotectants in the incidence study was based on the following rationale. One of the cryoprotectants generally used is a carbohydrate such as sucrose and sorbitol. We theorized that a high concentration of an easily fermentable carbohydrate might hinder the isolation of *C. botulinum* spores because the normal microbial flora could use the carbohydrate more readily. It could thus either outcompete any low levels of *C. botulinum* spores or rapidly lower the pH of the medium to inhibitory levels.

The amounts and types of cryoprotectants in surimi products are highly variable. Thus we used menhaden surimi without added cryoprotectants to establish a baseline on which effects of various combinations and levels of cryoprotectants on *C. botulinum* thermal resistance could be determined. The mean D-value in min at each temperature for *C. botulinum* type E spores in menhaden surimi is shown in Table 1. The D-values obtained in menhaden surimi at 73.9 and 76.7°C were within

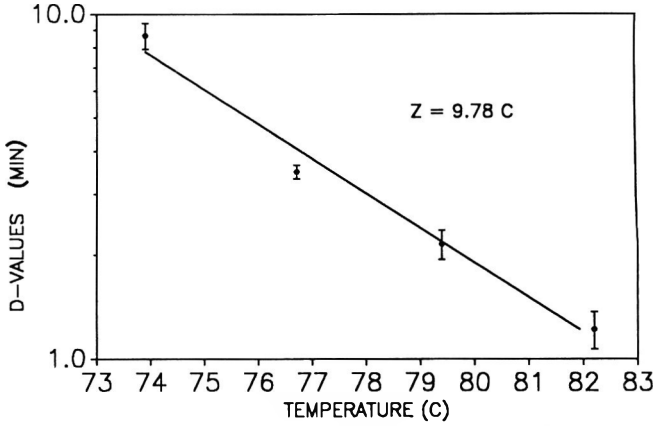

Fig. 1—Phantom thermal death time curve for Clostridium botulinum *type E spores in menhaden surimi.*

the range of reported values for type E in crabmeat, but those at 79.4 and 82.2°C tended to be slightly higher (Lynt et al., 1982). Similarly, our results agreed well with D-values obtained for *C. botulinum* type E in ground whitefish chubs (Crisley et al., 1968). A graph of the phantom TDT curve (Fig. 1) shows a z-value of 9.78°C, slightly higher than those generally reported for type E. This may have been due to surimi's unique characteristics, e.g., raw menhaden surimi is almost pure protein and has a very spongy texture. Another factor may have been the inclusion of a spore strain (MS-272) of unknown heat resistance in the original spore mixture.

Surimi gels are set by thermal processing. The product can be cooked with either a single- or a two-stage thermal process. The reported time/temperature conditions for single-stage cooking are generally between 40 and 60 min at 90°C (Chang-Lee et al., 1989; Douglas-Schwartz and Lee, 1988; Pacheco-Aguilar et al., 1989). Two-stage cooking is a common practice and generally uses a low-temperature stage to set the gel, followed by shorter high-temperature cooking time (Lee, 1984). Some minimal reported times for this second stage are 10 min at 90°C (Boye and Lanier, 1988) and 15 min at 88°C (Yoon et al., 1988). However, those reports did not indicate whether the time/temperature conditions were for the internal temperature (cold point) of the surimi. Most appeared to be for the external temperature applied for the specified time. Without adequate heat penetration data, comparisons for overall lethality of the processes are an estimation.

Thermal processing times and temperatures required to destroy *C. botulinum* spores vary for individual foods. Conditions for surimi are often modified to obtain desired textural and sensory characteristics. Our data indicated that minimal second-stage processing times would provide at least a 50D process for *C. botulinum* type E spores. However, our findings cannot be extrapolated into conclusions regarding the proteolytic and other nonproteolytic strains of *C. botulinum*. We suggest, therefore, that when changes are being considered to minimize thermal processing times and/or temperatures, the minimum requirements necessary to provide an adequate margin of safety should be considered.

CONCLUSIONS

ALTHOUGH the incidence of *C. botulinum* type E spores in surimi is low (1.2%), such spores have been isolated from raw menhaden surimi. Therefore, the manufacturers of surimi must consider the safety hazard and ensure destruction of *C. botulinum* spores. The thermal resistance of *C. botulinum* type E spores was slightly greater for menhaden surimi at the higher temperatures tested than for those previously reported. This is important when adequate thermal processes for menhaden surimi production are being determined. If second-stage cooking

—Continued on page 1592

Antioxidants to Preserve Rockfish Color

D.H. WASSON, K.D. REPPOND, and T.M. KANDIANIS

ABSTRACT

Thornyhead rockfish, *Sebastolobus alascanus*, were treated with mixed tocopherols, alone or plus ascorbic acid (TCAA), butylated hydroxytoluene (BHT) and sodium erythorbate to preserve red skin color during frozen storage. While all treatments significantly improved color retention compared to the control, at 4 mo the TCAA treated fish had significantly higher red color scores than any other treatment group, as measured using the CIE L*a*b* scale. The TCAA dip also appeared to enhance the red color compared to pre-treatment values.

Key Words: fish, rockfish, color, stability, antioxidants

INTRODUCTION

FISH SPECIES belonging to the genera *Sebastes* and *Sebastolobus*, commonly known as rockfish, comprise a comparatively small but commercially important component of the Alaskan fishery. Pacific ocean perch, *Sebastes alutus*, and thornyhead rockfish, *Sebastolobus alascanus*, are valuable commodities on the Japanese market, where the retail value is a direct function of skin color. The carotenoids astaxanthin and tunaxanthin are primarily responsible for the bright red hues of the rockfish (Tsukuda and Amano, 1966; Simpson, 1982). Astaxanthin is extremely unstable. Carotenoid degradation has long been recognized to proceed in analogous fashion to lipid degradation, i.e., by autoxidation (reaction with atmospheric oxygen, $^3O_2$) and/or photosensitized oxidation (reaction with singlet oxygen,$^1O_2$) (Frankel, 1985). Tsukuda and Amano (1967, 1968) also suggested that carotenoid degradation may be linked to lipoxygenase activity in the skin. While autoxidation of carotenoid pigments in fish has not been well studied, butylated hydroxytoluene (BHT) has been the antioxidant most extensively used in rockfish dips and glaze water to terminate the free radical reaction. However, research on prevention of lipid oxidation in fish oils (Khayat and Schwall, 1983) and other food commodities (Klaui, 1976) suggests that α- and γ-tocopherols may be more effective free radical chain terminators than BHT. Further, synergy of γ-tocopherol with ascorbic acid or ascorbyl palmitate has been shown to effect a several fold increase in antioxidant activity compared to BHT in animal fats (Klaui, 1976; Cort, 1974). For prevention of oxidation by means of $^1O_2$, α-tocopherol has proven to be the more effective tocopherol when added to soybean oil along with ascorbyl stearate (Yamauchi et al., 1981). The tocopherol regenerating activity of ascorbic acid is well documented (Packer et al., 1979, Yamauchi et al., 1981). Used alone, ascorbic acid also functions as an effective scavenger of $^3O_2$. Where the sharp acidic taste of ascorbic acid is a limiting factor, the epimer erythorbic acid has been used to retard oxidation in fish oils (Santos and Regenstein, 1990). In light of public concern over the health risks of BHT (Branen, 1975) our study was designed to evaluate the effectiveness of several alternative antioxidants for the preservation of the red skin color of the rockfish.

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MATERIALS & METHODS

Fish sampling and handling

Two hundred thornyhead rockfish, *Sebastolobus alascanus,* from the Gulf of Alaska were selected from a commercial trawl of about 907 kg in Kodiak, AK. The fish were less than 24 hr post-capture, and had been layered in ice since capture. Whole fish were chosen for uniformity of size (0.5 to 1 kg) and color (see Table 1 for pre-treatment a* values), then randomly divided and tagged individually into 5 lots of 40 fish. Each fish was measured in three locations for skin color before treatment using a Minolta Chroma Meter II Reflectance in the L*a*b* measuring mode to serve as a pre-treatment reference for changes during later storage and to determine if there were significant differences in pre-treatment mean L*,a* and b* values among groups. The three locations chosen to minimize errors in making repeat measurements at later sampling times and to serve as representative of the head, middle and tail regions, were respectively: (1) immediately posterior to the gill cover and dorsal to the pectoral fin, (2) immediately posterior to the pectoral fin and ventral to the lateral line, and (3) immediately anterior to the caudal fin.

Antioxidant treatment

Fish from each lot were then subjected to one of the following 5 treatments: (1) Control, no antioxidant, (2) 0.3% mixed tocopherols, of which about 80% were nonalpha forms ("Covi-Ox T-30P," Henkel Corporation, LaGrange, IL), (3) TCAA, 0.3% mixed tocopherols (Covi-Ox T-30P) + 0.03% pure ascorbic acid (Nature's Bounty, Inc., Bohemia, NY), (4) 0.05% BHT ("Sustane Emulsion-T," Nikki-Universal Co., Ltd., Tokyo, Japan), and (5) 0.05% sodium erythorbate ("Fish Glaze," Specialty Foods, Inc., Richmond, CA). "Covi-Ox T-30P" was chosen as a source of mixed tocopherols since the formulation incorporates a dextrin carrier that facilitates dispersion in water. "Sustane Emulsion-T," (a patented formula containing 10% BHT and 90% edible oils) was chosen as a source of BHT because it is highly water soluble and the only form of BHT currently used for fish. The final concentration of BHT, 0.05%, was obtained by preparing a 0.5% "Sustane Emulsion-T" solution. Sodium erythorbate was likewise obtained from a commercial formula containing inert ingredients in addition to the antioxidant. The Fish Glaze was prepared to yield a solution with a final erythorbate concentration of 0.05%. All antioxidant solutions were prepared with distilled water and fish in groups 2–5 were dipped once in the respective solution then frozen on trays at −18° C. Control fish were frozen without further treatment. The following morning the fish from all groups were glazed twice in a 0.5% solution of corn syrup solids ("Dry-Sweet Granulated Corn Syrup Solids," Hubinger-Heinz, Keokuk, IA). The glaze weight as a percent of total product weight was 3.0%. The glazed fish were double bagged in plastic inside wet-lock-boxes for further frozen storage at −18° C.

Color evaluation

Two days after treatment (1 day after glazing), 10 fish from each treatment group were removed from the freezer, allowed to thaw for 8 hr without exposure to light, then measured for L*a*b* values. Color values for each fish were then compared to the pre-treatment L*a*b* values obtained for the same fish, to compute difference values, (e.g., $\Delta L^* = L^*_{t(i)} - L^*_{t(3)}$). This procedure was followed after 1, 2 and 4 mo frozen storage.

Statistical analysis

Mean L*,a*, and b* values for each group of 40 fish pre-treatment and each group of 10 fish sampled post-treatment as well as mean changes in L*, a*, and b* compared to pretreatment values (ΔL*,

Table 1—Mean a values of rockfish treated with different additives and held in frozen storage*

Additive	PRE[c]	Storage time, Months[f] 0	1	2	4	ANOVA F P
None	14.9[a]	12.9[bAB]	11.6[bA]	8.3[cA]	4.4[dA]	47.88
	±3.9	±4.2	±4.0	±4.8	±2.5	0.000
Tocopherol	15.1[a]	12.9[bAB]	16.1[aB]	15.4[aC]	13.0[bB]	5.308
	±3.9	±2.9	±3.7	±3.3	±3.2	0.000
Tocopherol + Ascorbic acid	14.9	14.6[BC]	15.5[B]	16.6[C]	15.4[C]	1.631
	±3.8	±3.3	±3.1	±2.7	±3.8	0.167
Sodium erythorbate	14.0	12.6[A]	13.3[A]	13.4[B]	12.4[B]	1.254
	±4.5	±4.1	±3.2	±4.1	±4.5	0.289
BHT	14.7[a]	15.0[aB]	16.0[aB]	13.0[bB]	11.3[bB]	7.324
	±4.2	±3.7	±3.7	±3.2	±3.3	0.000
ANOVA						
F	1.247	2.696	9.096	22.28	41.72	
P	0.290	0.033	0.000	0.000	0.000	

[a-d] Means within a row not sharing a common letter were significantly different ($p < 0.05$).

[ABC] Means within a column not sharing a common letter were significantly different ($p < 0.05$).

[e] Pre-treatment means were computed for all 40 fish in each group combining values for head, middle and tail locations.

[f] "0" storage time denotes 1 day after glazing; "1", "2" and "4" denote 1, 2 and 4 mo, respectively, after treatment. Means at 0, 1, 2 and 4 mo were computed for 10 fish at each sampling time, combining values for three locations on each fish.

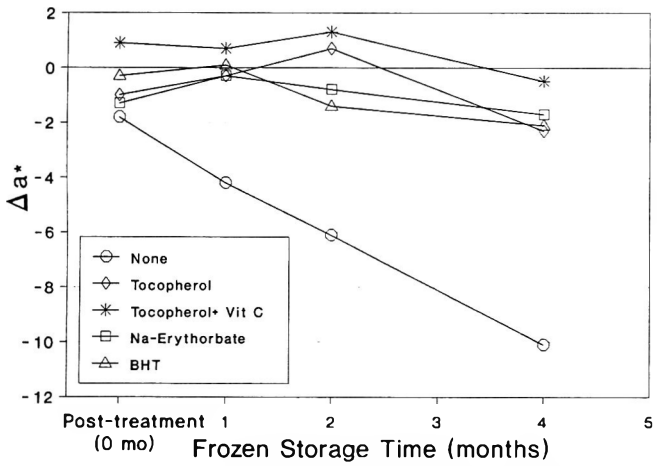

Fig. 1—Changes in △a with treatment and storage time. "0" denotes 1 day after glazing. Values represent means of △a* values for 10 fish, computed by comparing a* values at the given sampling time with a* values for the same fish pre-treatment. All three locations were combined.*

△a*, and △b*, respectively) were computed and analyzed using analysis of variance and least significant difference tests (Norusis, 1983). Each color component was also analyzed both as a function of the mean value for all locations on each fish and as a function of the mean value at each location, e.g., head, middle or tail.

RESULTS & DISCUSSION

COLOR VALUES obtained using the CIE L*a*b* scale correlated well with informal visual observations. The a* scale, measuring color gradation toward red (positive a* value) and green (negative a* value) was chosen as most representative of the change occurring in rockfish skin pigment with time. No significant differences were found in mean a* values between groups prior to antioxidant treatment and freezing (Table 1). At the end of 4 mo frozen storage, however, significant differences were noted. The TCAA group appeared distinctly redder than any other treatment group, and final a* values were significantly different ($p < 0.05$) (Table 1). However, there were no significant differences in measured redness of rockfish among the tocopherol without ascorbic acid, BHT and erythorbate groups. The control rockfish, on the other hand, had lost almost all red color after 4 mo despite double glazing with the corn syrup solids solution. An observable loss of redness was apparent only 2 days after freezing. The a* = 14.9 ± 3.9 pretreatment deteriorated to a* = 4.4 ± 2.5.

Figure 1, which charts the *changes* in mean a* values calculated at each sampling time compared to the mean pre-treatment a* values for the same lot of 10 fish, indicates that the TCAA dip had an enhancing effect on skin color. Only rockfish in that group had a positive mean △a* value (+0.9 ± 3.0) as a result of treatment (Fig. 1). Furthermore, there were no significant changes in the △a* at any later storage time, indicating full protection of the pigment in addition to color enhancement by the TCAA combination. The initial loss of

Table 2—Effect of different additives on △L and △b* values of rockfish with storage time[e]*

Additive	△L* Storage time, Months 0[f]	1	2	4	ANOVA F P	△b* Storage time, Months 0	1	2	4	ANOVA F P
None	−1.6[a]	3.5[b]	2.9[bA]	5.0[bA]	13.24	−0.6[aAB]	0.6[ab]	2.7[b]	−0.8[a]	4.025
	±3.6	±4.0	±3.7	±5.4	0.000	±3.8	±4.2	±4.2	±5.1	0.009
Tocopherol	−0.1[a]	2.2[bc]	3.2[bcA]	0.6[abB]	4.107	−0.5[AB]	−0.3	1.1	1.4	1.634
	±4.4	±4.0	±4.4	±3.4	0.008	±2.8	±5.6	±4.3	±3.7	0.185
Tocopherol +	−0.6[a]	2.6[b]	2.1[bA]	1.9[bB]	4.697	0.4[BC]	0.5	0.7	1.5	0.470
Ascorbic acid	±3.7	±3.2	±3.5	±3.9	0.004	±4.3	±4.3	±3.0	±3.9	0.704
Sodium	0.0	1.9	−0.2[B]	0.0[B]	1.577	−1.7[aA]	−0.3[a]	0.0[a]	2.3[b]	4.491
erythorbate	±4.2	±4.3	±4.8	±3.8	0.199	±3.8	±3.6	±4.8	±5.0	0.005
BHT	−0.2	1.8	1.3[AB]	1.5[B]	1.585	1.4[C]	0.0	0.7	0.3	0.552
	±3.3	±3.7	±4.9	±2.9	0.199	±3.4	±4.6	±3.9	±5.6	0.648
ANOVA										
F	0.879	0.939	2.954	7.262		3.032	0.265	1.780	2.023	
P	0.478	0.444	0.022	0.000		0.020	0.900	0.136	0.094	

[a-d] Means within a row which do not share a common letter were significantly different ($p<0.05$).

[A-C] Means within a column which do not share a common lower case letter were significantly different ($p<0.05$).

[e] △ values for head, middle and tail locations were combined. △L* and △b* values represent the means of 10 fish at each sampling. △ values were computed by comparing values at 0,1,2 and 4 mo to pre-treatment values for the same fish.

[f] "0" storage time denotes 1 day after glazing (i.e., 2 days after treatment and freezing).

Table 3—Mean L and b* values of rockfish treated with different additives and held in frozen storage*

Additive	L*	b*					ANOVA F
	PRE[d]	PRE	0 mo[e]	1 mo	2 mo	4 mo	P
None	46.8^{A}	7.7^{a}	7.8^{aBC}	9.4^{bB}	9.4^{bB}	6.2^{aA}	2.953
	±3.8	±4.9	±3.7	±3.1	±4.1	±3.4	0.021
Tocopherol	47.6^{AB}	8.0^{b}	5.7^{aA}	8.7^{bB}	9.8^{bB}	9.7^{bC}	4.569
	±3.8	±4.8	±3.1	±4.3	±3.8	±4.0	0.001
Tocopherol + Ascorbic acid	47.5^{AB}	8.3^{ab}	7.2^{aABC}	9.0^{bcB}	9.5^{bcB}	10.5^{cC}	2.929
	±3.7	±4.8	±3.7	±3.3	±2.8	±3.5	0.022
Sodium erythorbate	49.2^{C}	6.9	6.0^{AB}	6.6^{A}	6.9^{A}	8.6^{BC}	1.384
	±4.2	±5.3	±3.7	±3.2	±3.9	±4.0	0.240
BHT	48.0^{B}	7.5	8.2^{C}	8.4^{B}	7.9^{AB}	7.7^{AB}	0.359
	±4.0	±5.2	±3.9	±3.1	±3.5	±3.7	0.837
ANOVA							
F	5.811	1.365	2.810	3.086	3.394	6.017	
P	0.000	0.245	0.028	0.018	0.011	0.000	

[abc] Means within a row not sharing a common letter were significantly different ($p < 0.05$).

[ABC] Means within a column not sharing a common letter were significantly different ($p < 0.05$).

[d] Pre-treatment means were computed for all 40 fish in each group. Values for head, middle and tail locations were combined.

[e] "0 mo" storage time denotes 1 day after glazing; "1 mo" "2 mo" and "4 mo" denote 1, 2 and 4 mo, respectively, after treatment. Means at 0, 1, 2 and 4 mo were computed for 10 fish at each sampling time, combining values for 3 locations on each fish.

redness observed in the control group continued at a significant rate, reaching $\triangle a^* = -10.1 \pm 3.9$ at 4 mo. Although negative $\triangle a^*$ values were observed immediately post-treatment in the tocopherol and erythorbate groups, those values were not significantly different from the $\triangle a^*$ values observed at 4 mo. On the other hand, the 4 mo $\triangle a^*$ for rockfish in the BHT treated group (-2.1 ± 3.1) was significantly different from the $\triangle a^*$ immediately post-treatment (-0.3 ± 3.0).

Significant differences in pre-treatment L* values among groups (Table 3) invalidated comparison of mean L* values among groups at any later storage time, although the range in pre-treatment L* values was small, 46.8 ± 3.8 to 49.2 ± 4.2. Consequently, the ΔL^* values (Table 2) were considered more meaningful for analysis of differences among groups at 1, 2, and 4 mo. At 4 mo, the non-treated (control) rockfish had the highest $\triangle L^*$ and there were no significant differences among the treated samples. Increases in L* generally indicate the onset of freezer burn (unpublished data). However, since all treated groups at 4 mo for all locations combined had lower $\triangle L^*$ values than the control, (despite glazing) pigment loss appears also to be a factor.

There were no significant differences among groups in pre-treatment mean b* values, which measure the chromaticity scale from yellow (positive Δb^*) to blue (negative Δb^*). Comparison of mean b* values (Table 3) indicated significant differences between 0 and 4 mo in the 2 tocopherol groups. However, the Δb^* values (Table 2), which measured the change in mean b* values at 1, 2 and 4 mo (compared to pre-treatment mean b* values for the same 10 fish) indicated that only the erythorbate treated group changed significantly in that respect as a function of storage time.

The data (Fig. 1) suggest a possible mechanism for pigment degradation in rockfish. The antioxidant properties of tocopherols can be attributed to their effectiveness in repairing free radicals as well as their capacity to quench $^1O_2$. In research with vegetable oils and animal fats, the tocopherols have been shown repeatedly to be more effective terminators of free radical reactions than BHT. ΔA^* values in our study likewise showed that while there was no significant difference between the 0 and 4 mo Δa^* values for tocopherol treated fish, (-1.0 ± 2.0 and -2.3 ± 2.0) the Δa^* values for the BHT group began to decrease significantly between 1 and 2 mo. In addition, there was no significant difference in Δa^* values between the tocopherol and the erythorbate treated groups at 4 mo, (-2.3 ± 2.0 and -1.7 ± 2.7) respectively. Since erythorbate functions as a $^3O_2$ scavenger, it appears that tocopherol's role in the prevention of pigment degradation may be that of electron donor to free radicals generated by reaction with atmospheric oxygen. Singlet oxygen probably is not implicated under the conditions simulated in our study.

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Mention of trade names or commercial firms does not imply endorsement by the National Marine Fisheries Service, NOAA.

Factors Affecting Separation of Low Fat Flesh from Fatty Fish by Cryo-shattering

YOSHIO HAGURA and HISAHIKO WATANABE

ABSTRACT

Our previous report on separating low fat flesh from fatty fish by cryo-shattering indicated that at appropriate temperatures, fat content in particles increased with particle size. The effect was temperature dependent; that effect of low temperature on fracture stress and elastic modulus of model fish flesh was further examined at −60°C to −196°C. The fish flesh showed abrupt changes in compression and tensile fracture stress at about −90°C and −150°C; such changes strongly affected fat content-particle size relations (FCPSR). Using measured fracture stress and elastic modulus values, FCPSR was successfully simulated when Bond's equation was linked with an empirical equation to estimate work index values.

Key Words: fish, fatty, cryogenics, shattering, low-fat

INTRODUCTION

WHEN AGGLOMERATED foodstuff is cryo-shattered at the appropriate low temperature, individual granules separate (Watanabe et al., 1987). Cryo-shattering followed by cryo-sieving of mackerel and sardine allowed separation of a low fat fraction from those fatty fish (Hagura et al. 1989). The separation is based on the fact that at an appropriate temperature (−60°C ~ −80°C), most fat is excluded from smaller-sized shattered particles. The effect was temperature dependent. At −196°C, the fat content in particles increased with decreasing particle size (Hagura et al., 1989).

We have now examined experimentally the effect of low temperature on fracture stress and elastic modulus, the dominant factors affecting size of cryo-shattered particles. Our objective was to elucidate the fat content-particle size relationship at cryo-shattering temperatures ranging from −60°C to −196°C. Rittinger's, Kick's, and Bond's equations are well known for describing particle size and energy input in size reduction processes. Among these, Bond's equation, Eq. (1), is the best for practical use in correlating data in size reduction (Bond, 1952):

$$P = 10\,\alpha W_i\,(1/\sqrt{D_2} - 1/\sqrt{D_1}) \qquad (1)$$

Where: P (J/kg) is the energy for shattering a material to reduce size from D_1 to D_2 μm. W_i, the work index, represents a characteristic resistance of the material against shattering. The coefficient α depends on the type of shattering machine: α = 1 for ball mill and 1.34 for hammer mill (Snow, 1984). According to Eq. (1), the work index determines the size of the shattered particle when the shattering machine is operated with constant energy input.

Using a dimensional analysis, Yashima et al. (1970) successfully correlated work index with mechanical properties: density, ρ, elastic modulus, E, compressive fracture stress, σ_c, tensile fracture stress, σ_t, and ratio of specific surface area of intact specimen to that of fractured particles, R.

$$W_i = 0.623\,\rho^{-1.00}\,E^{0.35}\,\sigma_c^{0.15}\,\sigma_t^{0.50}\,R_c^{-0.09}\,R_t^{-0.48} \qquad (2)$$

subscript c = compressive and t = tensile.

In our current study, the objective was to determine the work index of fish flesh from mechanical properties of model fish flesh with varied fat content. Such work index values could then be substituted into Eq. (1) to simulate curves of shattered particle size vs fat content.

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MATERIALS & METHODS

Test pieces

Alaska pollack surimi (Taiyo Gyogyo Co.; SA grade) was mixed with varied amounts of cod oil (Yakuro Seiyaku Co.) for a model fish flesh. Surimi stored at −30°C was thawed at 5°C for 12 hr before use. The mixture using cod oil (0,10,20,40,50% weight basis) was minced with a blender mill (6,000 rpm; 5 min), and poured into aluminum tubes (20 mm i.d. × 20 mm) with the bottoms covered with polyethylene film. Tubes filled with surimi were wrapped on top in polyethylene film and placed in a freezer at −30°C for 12 hr. Cylinders removed from the tubes were used as test pieces for compression.

Dumbbell-shaped test pieces (cross section of test portion: 10 mm × 10 mm) were used for tension tests. We prepared 10 mm thick frozen plates of model fish flesh with varied fat content at −30°C in a freezer. The dumbbell-shaped test pieces were cut out of the frozen model fish flesh plate in accordance with Japanese Industrial Standards for testing industrial materials (Fig. 1; JIS, 1981). 100% fat content test pieces were prepared using cod oil in a similar manner. Cylindrical test pieces were prepared using aluminum tubes, dumbbell-shaped test pieces were cut out of frozen cod oil plates.

Apparatus and Procedure

A testing machine (Model UTM-4-200, Toyo Baldwin Co.) was used for uniaxial compression tests as well as for uniaxial testion tests (Fig. 2). A test piece A, cooled with cold nitrogen gas evaporating from liquid nitrogen contained in a Dewar vessel B, was compressed between bearing plates C_1 and C_2, or pulled between jaws D_1 and D_2, at a constant rate of deformation (4.0 mm/min). Varying the evaporation rate of liquid nitrogen and/or distance between the test specimen and the surface of liquid nitrogen allowed control of the temperature of the test piece. The evaporation rate was regulated by controlling the current to immersion heater H. The lower bearing plate C_2 or the upper jaw D_1 was connected to load cell F (Model TM-200L, Orientec Co., capacity: 200 kgf). The signal from the load cell was amplified through a strain amplifier. The displacement of the test piece was detected by a digital strain-meter (Model GS-332, Onosokki, discrimination: 0.001 mm). A load-deformation curve was recorded with an X-Y recorder E.

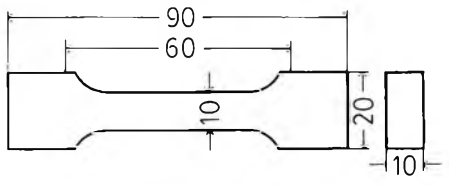

Fig. 1—Dumbbell-shaped test piece.

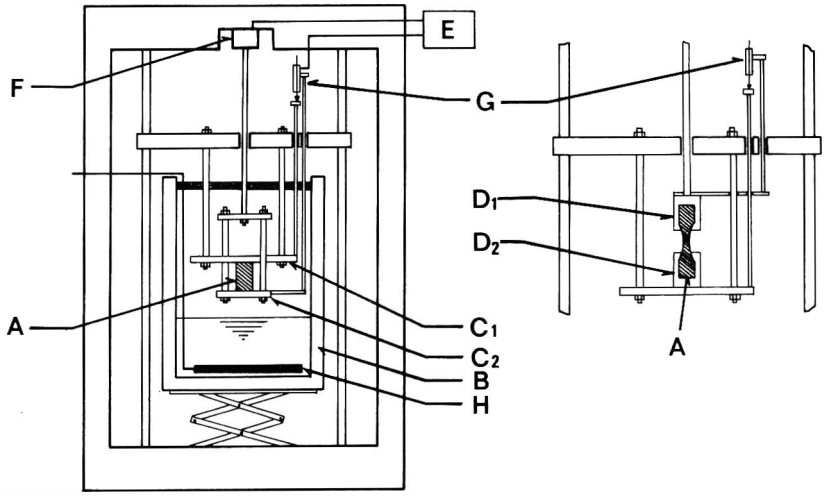

Fig. 2—Schematic diagram of apparatus.
(A) test piece, (B) Dewar vessel, (C) bearing plate, (D) jaw, (E) X-Y recorder, (F) load cell, (G) strain-meter, (H) immersion heater.

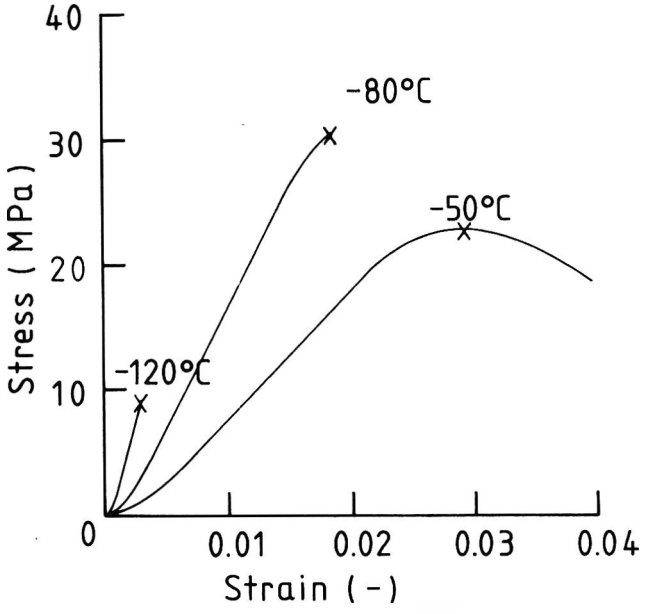

Fig. 3—Typical stress-strain curves of model fish flesh in compression.

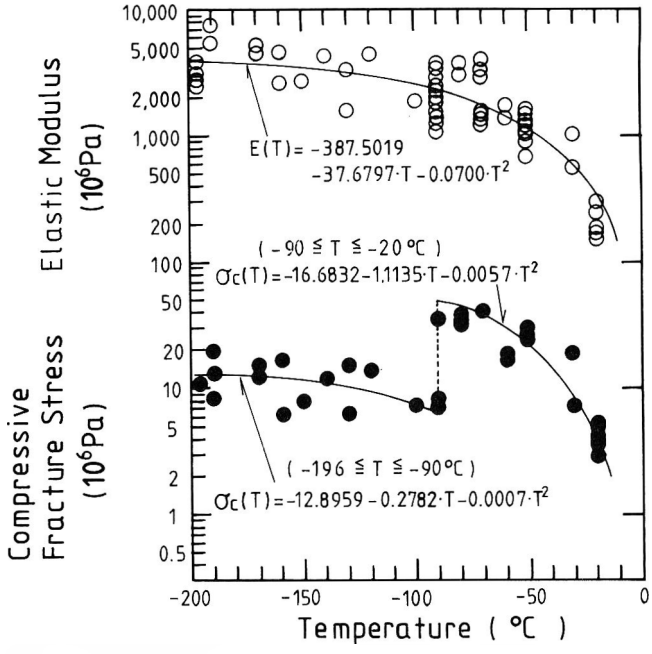

Fig. 4—Elastic modulus (○) and compressive fracture stress (●) of oil-free surimi sample at low temperatures. Solid lines denote best fit lines by a least squares regression.

A test piece, placed between the bearing plates or jaws which had been cooled in advance with cold nitrogen gas, was cooled to a preset test temperature between −10°C and −196°C. The test piece was cooled slowly and carefully at a rate of about 2.0°C/min to avoid cracking due to thermal shock.

Specific surface area

Specific surface area, the ratio of surface area of a body to its weight, was calculated from the dimensions and mass of the specimen. The surface area of particles from a fractured test piece was estimated by a well known method based on particle size distribution (Berry, 1950). That value for the fractured material was measured using sieve analysis by plotting the cumulative data on logarithmic probability paper (Hagura et al. 1989).

RESULTS & DISCUSSION

Elastic modulus of model fish at low temperature

Typical stress-strain curves for selected temperatures are shown in Fig. 3. At low temperatures, a sudden breakage was observed, while at high temperatures a gradual deformation took place. For slight deformations, the stress-strain curves approached straight lines. The slope of such straight lines gave the elastic moduli. The x marks in Fig. 3 indicate fracture stress.

Elastic moduli obtained from stress-strain curves in compression tests with cod oil-free surimi samples are plotted vs temperature in Fig. 4. The solid line through the data points represents the best fit line by a least squares regression. Elastic moduli on compression were not different from those under tension. In Fig. 5, we show the effect of temperature on elastic modulus of model fish flesh with varied fat content as well as that of the 100% cod oil sample.

The elastic modulus of model fish flesh and 100% cod oil (Fig. 5) increased considerably as temperature decreased throughout the range of temperatures tested. This temperature dependence of fish flesh moduli agreed well with that reported for orange juice ice (Watanabe et al., 1991). On the other hand, elastic modulus of pure materials generally increases

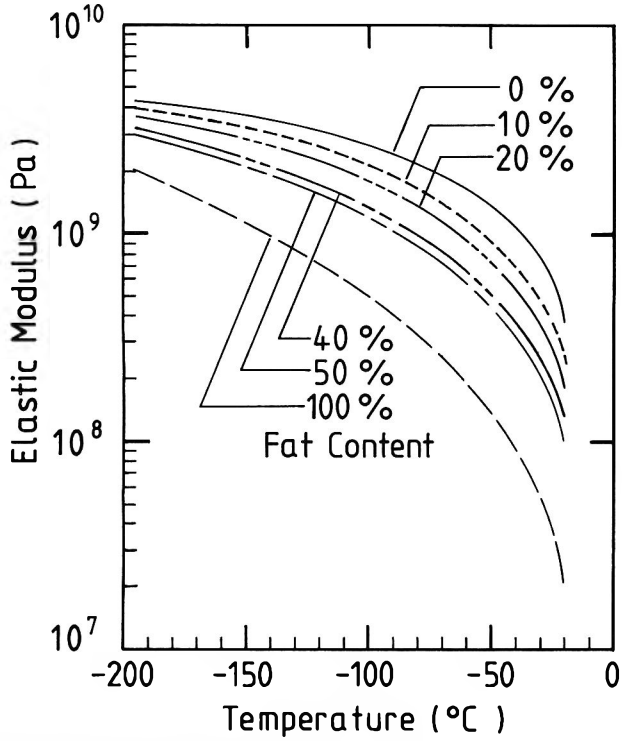

Fig. 5—Effect of low temperature on elastic modulus of model fish flesh of different fat contents.

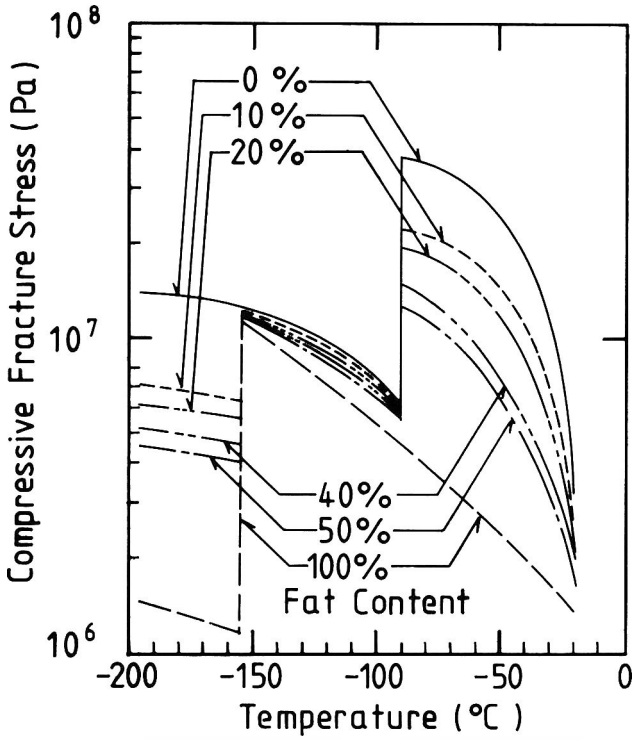

Fig. 6—Effect of low temperature on fracture stress in compression test of model fish flesh of different fat contents.

very gradually with increase in temperature (DiBenedetto, 1967). The elastic modulus of pure water ice, for example, is 6.0×10^9 Pa at −5°C (Gold, 1958) and 6.9×10^9 Pa at −196°C (Parameswaran and Jones, 1975).

Fracture stress of fish flesh at low temperature

When fracture stresses in compression tests with cod oil-free surimi samples were plotted vs temperature (Fig. 4) they

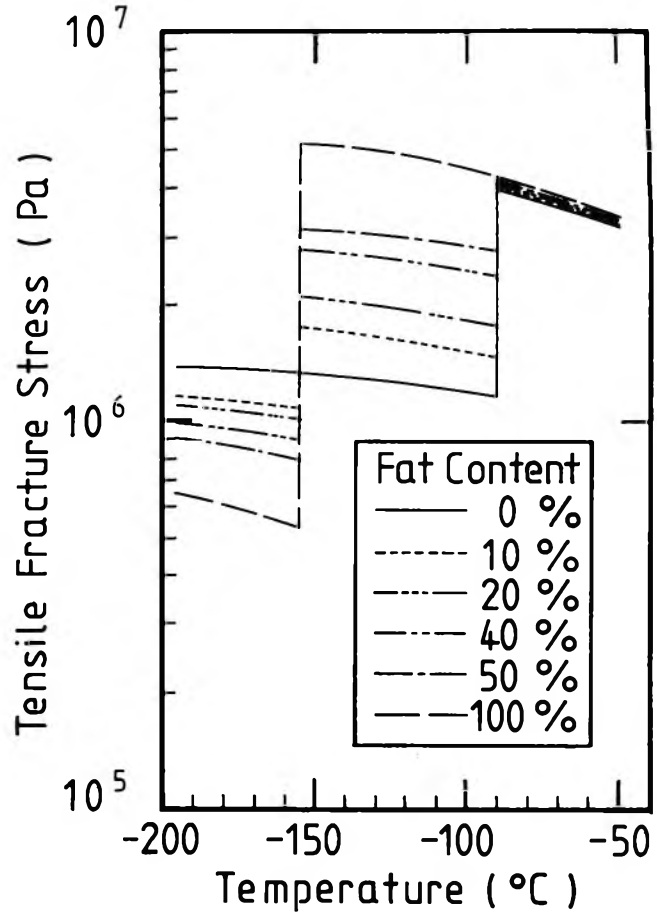

Fig. 7—Effect of low temperature on fracture stress in tension test of model fish flesh of different fat contents.

increased considerably as temperature decreased between −20°C and −90°C. The solid line in the figure represents the best fit line by least squares regression. There followed an abrupt drop from 40×10^6 Pa to 7×10^6 Pa at around −90°C. Fracture stress increased gradually as temperature decreased between −90°C and −196°C.

When fracture stresses in compression tests using samples with varied fat content were plotted vs temperature (Fig. 6) those of a 100% cod oil sample showed considerable increase as temperature decreased from −20°C until a sharp drop occurred at −155°C. Considering the fracture stress of surimi sample minced with cod oil, at −90°C and −155°C, sharp decreases occurred. Each of these temperatures also corresponded to the temperature at which cod oil-free sample or 100% cod oil sample showed a sharp drop in fracture stress. At temperatures higher than −90°C or lower than −155°C, we observed that the lower the fat content the larger the compression fracture stress. On the other hand, compression fracture stresses were nearly unique values regardless of fat content when the test temperature was between −90°C and −155°C.

Fracture stresses in tension are shown in Fig. 7. Tensile fracture stress also had two temperatures at which sharp stress drops occurred. The two temperatures agreed with those of compression fracture stress. An inverse relationship was seen in the temperature range −90°C to −155°C, where the lower the fat content, the smaller was the fracture stress.

Calculation of work index

To calculate the work index using Eq. (2), the density and ratio of specific surface area were required in addition to elastic modulus and fracture stress. The density of the model fish flesh obtained by measuring its weight and volume was constant:

Table 1—Ratio of specific surface area measured in fracture test

Fat (%)	Temperature (°C) −60	−100	−150	−190
	R_c (Compression test)			
0	15.0	23.2	49.6	12.1
20	9.5	15.0	34.9	11.6
40	2.6	9.2	28.4	9.4
50	1.8	5.2	18.6	8.0
	R_t (Tension test)			
0	6.0	1.8	1.3	1.3
20	2.8	1.3	1.3	1.3
40	1.3	1.3	1.3	1.3
50	1.3	1.3	1.3	1.3

Table 2—Work index calculated from Eq. (2)

Fat (%)	Temperature (°C) −60	−100	−150	−190
	W_i (kJ/kg)			
0	8.4	8.9	11.1	14.2
20	9.7	10.2	13.0	10.6
40	13.6	11.9	14.4	9.4
50	12.8	12.9	15.0	8.8

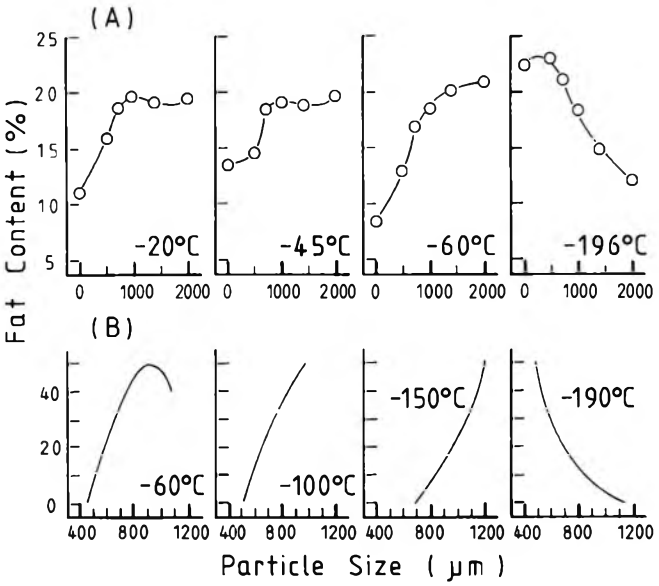

Fig. 8—Experimental results of fat content-particle size relationship using mackerel by Hagura et al. (1989) (A) and that predicted in this work (B).

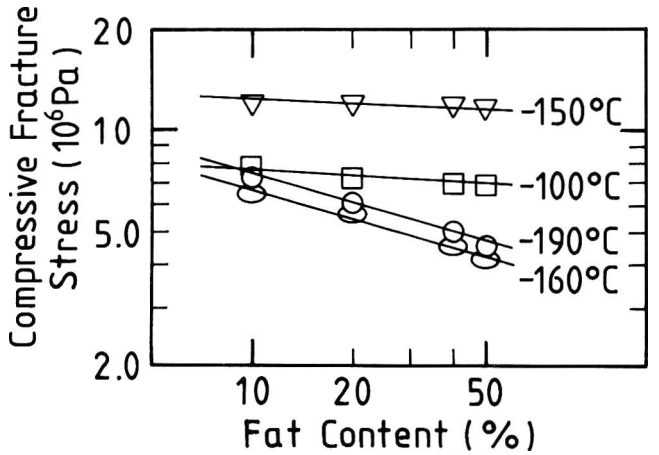

Fig. 9—Effect of fat content on compression fracture stress of model fish flesh.

1.0×10^3 kg/m^3, at −60°C to −190°C. Ratios of specific surface areas are listed in Table 1. R_c values varied considerably with temperature as well as fat content, while R_t values were constant.

Measured or estimated values for factors ρ, E, σ_c, σ_t, R_c, and R_t were substituted into the right-hand side of Eq. (2), to obtain the work index W_i at temperatures −60, −100, −150, and −190°C (Table 2).

Estimating particle size of cryo-shattered model fish flesh

In our previous report we separated low fat flesh from mackerel via cryo-shattering using a hammer mill equipped with 16 hammers and a screen with 5.00 mm opening operated at 2600 rpm (Hagura et al., 1989). Results of those experiments were reported as fat content-particle size curves at selected shattering temperatures (Fig. 8A).

In our current report, we attempted to simulate the fat content-particle size curves by substituting the calculated work index values (Table 2) into Eq. (1). The size of mackerel samples (40,000 μm) fed into the shattering machine in the previously reported experiment were used as the initial sample size, D_1.

The energy required to drive the shattering machine, P, may be estimated using an empirical energy chart described by Bond and Wang (1950). This chart enabled an estimate of the energy input required to crush a material of medium hardness when average size of feed and product were known. Assuming feed size of 40,000 μm and product size of 2,200 μm, we determined the energy input, P, of 4.70×10^3 J/kg.

The simulated particle size of cryo-shattered fish flesh plotted on the horizontal axis vs fat content (Fig. 8B may be compared with experimental results (Hagura et al., 1989) shown in Fig. 8A. The simulated fat content-particle size curves demonstrated that at −60°C the smaller the particle size the lower the fat content. This relationship reversed when the sample was cryo-shattered at −190°C.

Although Fig. 8B demonstrated clearly that use of Eq. (1) and (2) would produce results which correctly described shattered particle size as a function of fat content and temperature, the reverse effect in fat content-particle size relationship at −150°C was not explicitly shown. The main reason for that is explained as follows: When shattering something using shattering machine at constant power, and the size of material fed into the machine is constant, the size of shattered particles, D_2, may be given (Eq. (1)) as:

$$D_2 \propto W_i^2 \tag{3}$$

Discarding factors from Eq. (2) which have relatively low exponents and those which change very little in value with fat content:

$$W_i \propto E^{0.35}\, \sigma_c^{0.15}\, \sigma_t^{0.50} \tag{4}$$

Substituting Eq. (4) into Eq. (3):

$$D_2 \propto E^{0.70}\, \sigma_c^{0.30}\, \sigma_t^{1.00} \tag{5}$$

The effect of fat content on fracture stress and elastic modulus was determined using data at selected temperatures. In Fig. 9 and 10 the effects of fat content on fracture stress were greatly influenced by temperature of fracturing. The effect of fat content on fracture stress was:

$$-150\ °C < T < -90\ °C$$

$$\sigma_c \propto f^{-0.05} \tag{6}$$

$$\sigma_t \propto f^{0.35} \tag{7}$$

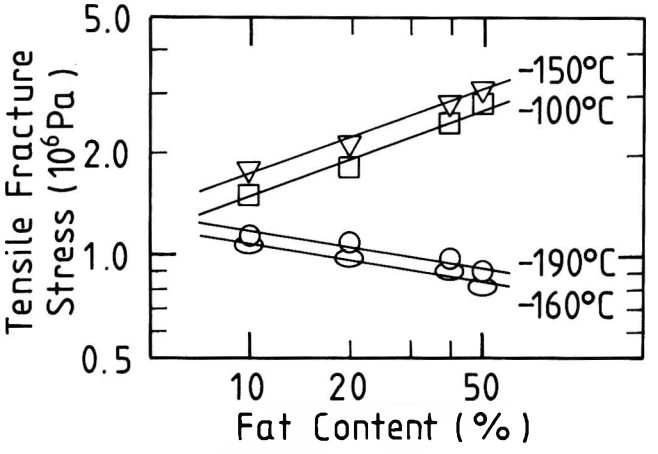

Fig. 10—Effect of fat content on tension fracture stress of model fish flesh.

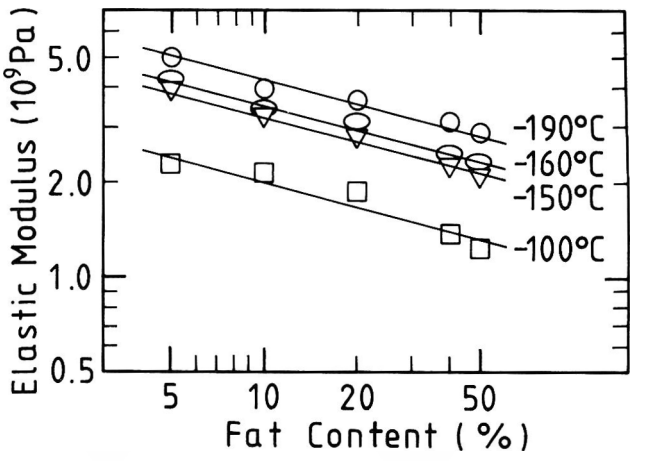

Fig. 11—Effect of fat content on elastic modulus of model fish flesh.

$$-196\ °C < T \leq -160\ °C$$

$$\sigma_c \propto f^{-0.28} \quad (8)$$

$$\sigma_t \propto f^{-0.16} \quad (9)$$

On the other hand, a log-log plot of elastic modulus vs fat content gave nearly a single slope of -0.26 regardless of temperature (Fig. 11).

$$E \propto f^{-0.26} \quad (10)$$

Substituting these relations into Eq. (5):

$$D_2 \propto f^{0.15} \qquad -150\ °C < T < -90\ °C \quad (11)$$

$$D_2 \propto f^{-0.43} \qquad -196\ °C < T \leq -160\ °C \quad (12)$$

The change in exponent from positive in Eq. (11) to negative in Eq. (12) signified the reversion in dependence of particle size to fat content at different ranges. The tensile fracture stress also exhibited a slope change from positive above -150°C to negative at lower temperatures.

CONCLUSIONS

BOND'S EQUATION was successfully used to simulate fat content-particle size curves in cryo-shattering fatty fish when it was linked with work index values estimated using an empirical correlating equation. Frozen fish flesh showed abrupt change of compression and tensile fracture stress at about -90°C and -150°C. This change strongly affected the shattered particle size. The reversion in dependence of particle size on fat content was mainly caused by the temperature dependence of tensile fracture stress in frozen fish flesh.

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Thermal Degradation of Paralytic Shellfish Poison

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ABSTRACT

Components of paralytic shellfish poison (PSP) were heated under various conditions, and examined for changes in toxicity and in HPLC and TLC behaviors. The thermal degradation of PSP components progressed as a first order reaction, at different rates depending upon component and temperature. A mixture of gonyautoxin 2 and gonyautoxin 3 when dissolved in water and heated at 100°, 110°, and 120°C (180 min) retained 39, 17, and less than 3%, respectively, of initial toxicity. A mixture of gonyautoxin 1 and gonyautoxin 4 was more thermolabile; i.e. it retained little toxicity when heated at 120°C for 60 min. HPLC and TLC analyses demonstrated that heating under those conditions converted PSP components into other substances.

INTRODUCTION

BIVALVES are often toxified with paralytic shellfish poison (PSP) that is produced by some species of dinoflagellates and blocks the sodium channel of the nerve system (Schantz, 1986). Contamination of commercially important shellfish with PSP poses serious problems to shellfish and related industries in Japan and other countries.

PSP is accumulated in the digestive gland of bivalves such as the scallop (Maruyama et al. 1983). Repetition of freezing and thawing resulted in migration of PSP from a highly toxic to a barely toxic tissue (Noguchi et al., 1984). Attempts have been made to detoxify the accumulated PSP in bivalves. Blogoslawski and Stewart (1978) reported that an ozone treatment enhanced the elimination of PSP from contaminated bivalves. Heat treatment was also effective to reduce PSP toxicity in shellfish. Prakash et al. (1971) reported that the total toxicity in a scallop was decreased by about 90% during canning. Noguchi et al. (1980a, b) also demonstrated that a significant reduction of toxicity in the Japanese scallop *Patinopecten yessoensis* occurred during retorting and that a slow but steady reduction of the remaining toxicity in canned scallop occurred during storage. These findings indicated that canning is a useful and practical means to make PSP-contaminated shellfish acceptable as food. The mechanism involved in reducing toxicity, however, is not clear. Our research was undertaken to examine heat-induced changes in toxicity and composition of PSP, as part of studies to elucidate the mechanism.

MATERIALS & METHODS

Materials

Specimens of the scallop *Patinopecten yessoensis* were collected from Ofunato Bay, Iwate Prefecture. Digestive glands were immediately excised, combined, transported in dry ice to the laboratory of Marine Biochemistry and kept below −20°C until used. Specimens of oyster *Crassostrea gigas* were collected from Senzaki Bay, Yamaguchi Prefecture, and immediately transported with ice to the Laboratory. The whole edible parts were excised, combined, and kept below −20°C until used.

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Mixtures of gonyautoxins 1 and 4 ($GTX_{1,4}$) and of gonyautoxins 2 and 3 ($GTX_{2,3}$) were prepared from the toxic scallop digestive glands according to the method of Onoue et al. (1981). A mixture of saxitoxin (STX) and neosaxitoxin (neoSTX) was prepared from a toxic crab *Zosimus aeneus* which was collected from Ishigaki Island, Okinawa Prefecture, by a method reported previously (Daigo et al., 1985).

Heating treatment

The freeze-dried samples of $GTX_{1,4}$ and $GTX_{2,3}$ were dissolved in distilled water, to give a final concentration of 200 mouse unit (MU)/mL. (One MU is defined here as that amount of toxin which kills a 20g ddY strain male mouse in 15 min after intraperitoneal injection, Kawabata, 1978). Three-mL aliquots of each toxin solution were placed in screw-capped vials, and heated at 100, 110, and 120°C for 180 min, in an autoclave. The temperature was recorded by type a Z9-CTF recorder® (Electro-laboratories, Copenhagen, Denmark). After heating, toxin solutions were immediately cooled in water, and subjected to toxicity assay and other analyses. The frozen scallop digestive glands (170 MU/g) and frozen oyster edible parts (15 MU/g) were partially thawed and homogenized using a blender. Five-gram aliquots were placed in screw-capped vials, and heated in the same manner as were the PSP solutions.

Extraction and partial purification of PSP from heat-treated shellfish homogenate

PSP was extracted from heated bivalve homogenates by ultrasonication with 2 volumes of distilled water for 30 min. The extract was centrifuged at $750 \times g$ for 15 min. A portion of the supernatant was assayed for toxicity by the mouse bioassay method (Kawabata, 1978).

The remaining supernatant was repeatedly defatted with dichloromethane. After evaporating the contaminating dichloromethane, the aqueous layer was filtered through a Diaflo YM-2 ultrafiltration membrane® (Amicon, Danver, MA) to cut off >1,000-daltons. The filtrate was chromatographed on a Bio-Gel P-2® (Bio-Rad Laboratories, Richmond, VA) column (95 × 2cm i.d.), using 0.03M acetic acid. Toxic fractions were combined, lyophilized, and subjected to analyses.

Toxicity

Toxicity was determined by a mouse bioassay method for PSP, using ddY strain male mice weighing 19–21g (Kawabata, 1978). Toxicity was expressed as means of triplicate determinations.

Identification of toxins

Toxins were identified by HPLC and TLC, based on co-chromatography with authentic PSP standards. HPLC analysis for PSP was carried out according to the method of Nagashima et al. (1987). Briefly, a reversed-phase ODS column (YMC AM-314®, Yamamura Kagaku, Kyoto, Japan; 30 × 0.6cm i.d.) was developed with 0.05M phosphate buffer containing 2mM 1-heptanesulfonic acid/methanol (99:1) for GTXs and (75:25) for STXs. PSP components were converted into fluorogenic substances by a periodate reagent, and monitored at 390nm with 336nm excitation.

TLC was conducted on 10 × 10cm Whatman LHP-K plates® (Whatman, Clifton, NJ) with a solvent system of pyridine: ethyl acetate: acetic acid: water (15: 5: 3: 4). Toxins were detected under UV light at 365 nm before and after plates were sprayed with 1% H_2O_2, and heated at 110°C for 5 min.

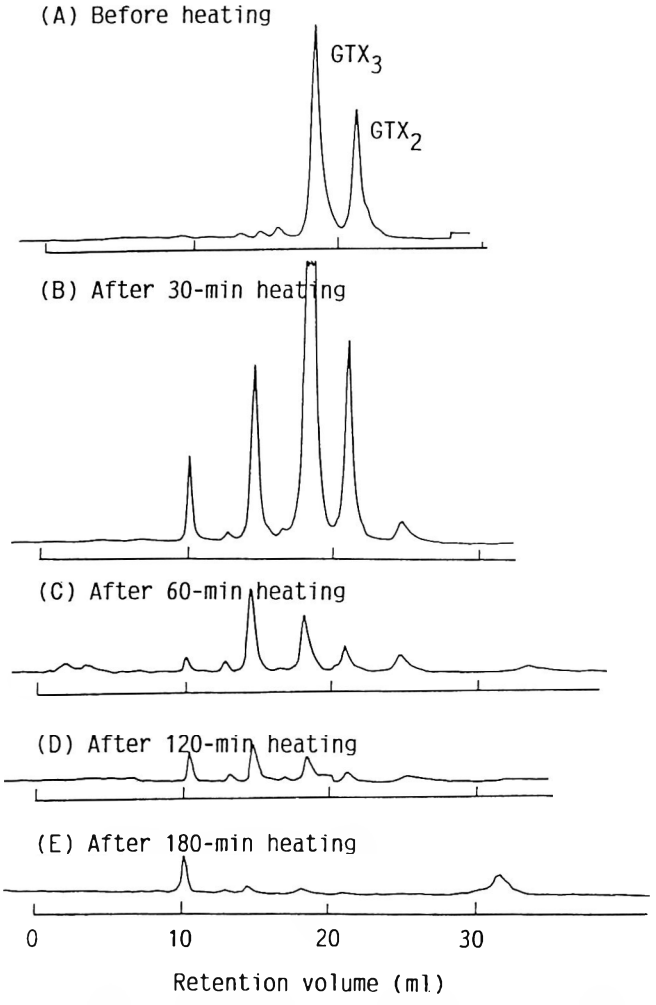

Fig. 1—HPLC of $GTX_{2,3}$ before and after heating in water at 120°C.

Table 1—Thermal degradation rates of PSPs dissolved in water, and of those contained in scallop digestive gland homogenates, as expressed by the slope of the first order plot

Sample	Heating temp	Slope*	
$GTX_{2,3}$	100°C	2.17 ± 0.43^a	(−0.940)**
	110	3.38 ± 0.56^a	(−0.929)
	120	9.66 ± 1.21^a	(−0.970)
$GTX_{1,4}$	100	3.32 ± 0.79^a	(−0.964)
	110	1.07 ± 0.14^b	(−0.960)
	120	3.38 ± 0.51^b	(−0.971)
Scallop digestive gland homogenate	100	3.86 ± 0.66^a	(−0.838)
	110	9.50 ± 2.05^a	(−0.990)
	120	2.72 ± 0.71^b	(−0.982)

* All values ± 95% confidence interval; ^{a} $\times 1 \times 10^{-3}$; ^{b} $\times 1 \times 10^{-2}$
** Correlation coefficient.

Statistical analysis

Linear regressions and correlation coefficients were calculated by the method of Snedecor and Cochran (1967). Reduction rates of toxicity were determined using a first order model.

RESULTS & DISCUSSION

THE TOXICITY of PSP decreased with prolongation of heating time and elevation of temperature. Linearity of the semilog plot of toxicity remaining versus heating time was observed, with a high correlation coefficient (Table 1). The toxicity of $GTX_{2,3}$ declined as a first order reaction with rates of $(-2.17 \pm 0.43) \times 10^{-3}$, $(-3.38 \pm 0.56) \times 10^{-3}$, and $(-9.66 \pm 1.21) \times 10^{-3}$, respectively, at 100, 110, and 120°C.

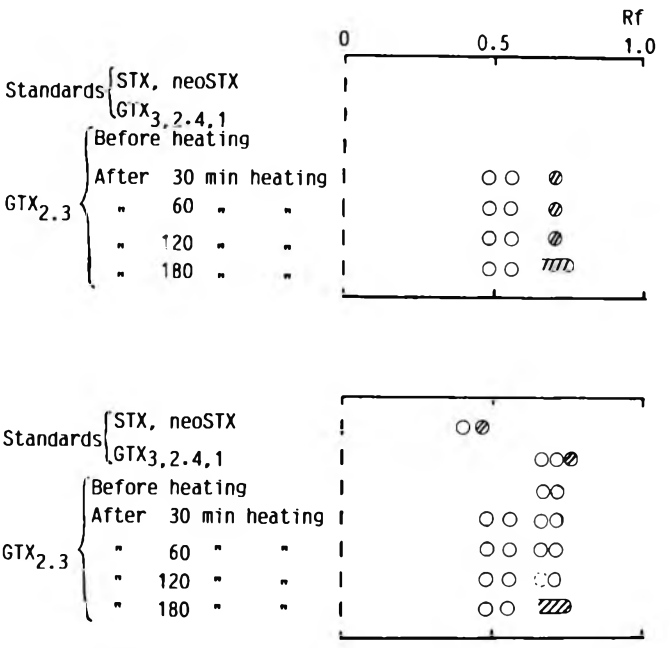

Fig. 2—TLC of heat-treated $GTX_{2,3}$ before (upper) and after (lower) spraying with H_2O_2 followed by 5-min heating at 110°C. $GTX_{2,3}$ was dissolved in water and heated at 120°C for indicated minutes and analyzed by TLC. Detection: UV at 365nm. ○ Blue fluorescence; ◎ greenish blue fluorescence

$GTX_{1,4}$ was more thermolabile than $GTX_{2,3}$. The thermal degradation rates of $GTX_{1,4}$ at 110 and 120°C were about three times higher than those of $GTX_{2,3}$.

The degradation of PSP contained in scallop digestive gland homogenate also progressed as a first order reaction. The degradation rates of scallop PSP were comparable to those for $GTX_{1,4}$. Scallop digestive gland specimens having an initial toxicity of 170 MU/g had a toxicity below 4 MU/g (the quarantine limit of PSP in Japan) when heated at 110°C for 180 min or at 120°C for 120 min.

HPLC analysis (Fig. 1) of $GTX_{2,3}$ toxins heated at 120°C for 30 min showed five peaks, two major ones corresponded to GTX_3 and GTX_2. Peaks corresponding to GTX_2 and GTX_3 were barely detectable after 180-min heating. Three peaks (e.g., V_R 10.1, 14.5, and 24.6 mL) appeared after 30 min-heating, and became smaller as heating time increased. TLC of $GTX_{2,3}$ heated at 120°C confirmed the results of HPLC (Fig. 2): Spots of GTX_2 (Rf 0.74) and GTX_3 (Rf 0.70) became faint with increasing heating time, and finally disappeared after 180 min, while three spots with Rf values of 0.49, 0.58, and 0.73 appeared on heating. Heated $GTX_{1,4}$ gave similar results to those of $GTX_{2,3}$ on HPLC and TLC analyses. Both GTX_1 and GTX_4 disappeared as heating time increased (data not shown).

Figure 3 shows HPLC patterns of the toxin from scallop digestive gland homogenates before and after heating at 120°C for 120 min. Before heating, the toxin consisted of GTX_{1-4} as the major toxin, along with STX as the minor. Those peaks disappeared on heating. This agreed well with results of the toxicity assay which showed that scallop digestive gland homogenate became almost nontoxic when heated under those conditions.

Heat-induced changes in toxicity of PSP contained in the edible part of oysters clearly differed from those for GTX standards and scallop digestive gland homogenate (Fig. 4). Toxicity was increased from the initial 15 MU/g to 30 MU/g by heating at 100°C for 30 or 60 min, but decreased linearly on further heating. Oyster PSP showed essentially the same patterns of toxicity change when heated at 110 and 120°C, but was more rapidly degraded than at 100°C.

Low-toxic, carbamoyl-N-sulfo PSPs such as GTX_5 and GTX_6

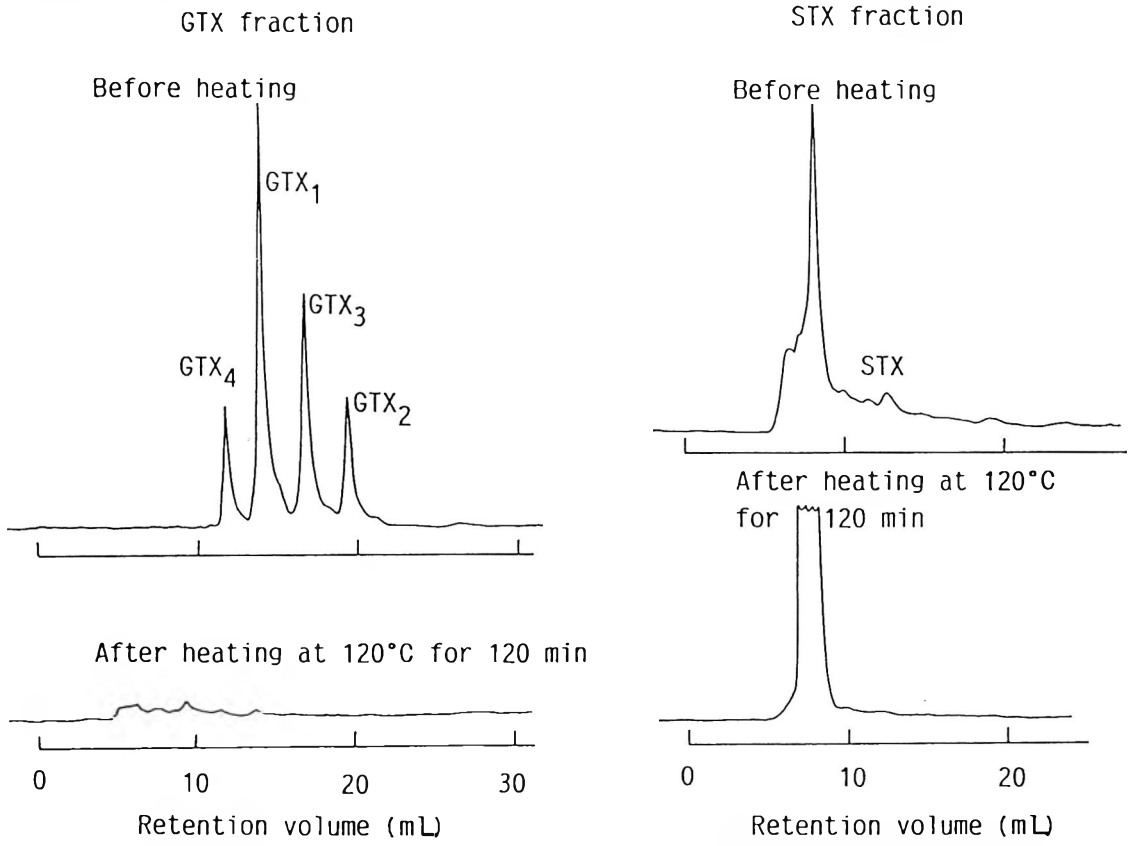

Fig. 3—HPL of GTX and STX fractions from a toxic scallop digestive gland homogenate, before and after heating at 120°C for 120 min.

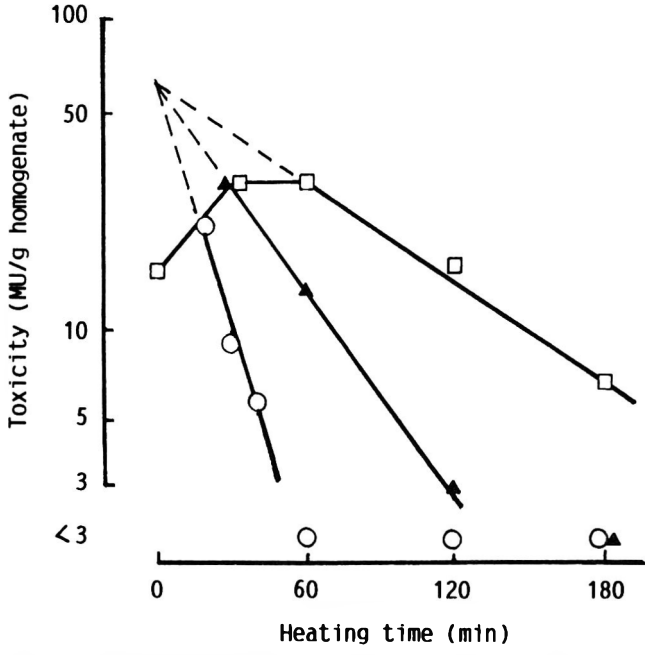

Fig. 4—Thermal degradation of PSP contained in an oyster homogenate at 100 (—□—), 110 (—▲—), and 120°C (—○—).

are easily converted into highly-toxic carbamoyl counterparts on mild acid hydrolysis, resulting in a remarkable increase in toxicity (Kobayashi and Shimizu, 1981; Harada et al., 1982; Nishio et al., 1982; Hall and Reichardt, 1984). The transient increase of toxicity on heating could have been caused by this mechanism. The oyster PSP consisted of PXs along with GTX_5 and GTX_6 (Fig 5). These three components disappeared on heating at 100°C for 60 min (Fig. 5). A peak (V_R 14.5 mL) appeared with GTX fraction and another peak (V_R 10.9mL) with STX fraction (Fig. 5). The GTX peak was not identical with any known GTX components, but with an unknown component derived from $GTX_{2,3}$ (Fig. 1). The peak with the STX fraction did not correspond to neoSTX nor STX. The 60-min heating at 120°C eliminated both peaks (Fig. 5), making the oyster homogenate nontoxic.

The quarantine limit of PSP is 4 MU/g edible part in Japan, (almost equivalent to that in the U.S.A. and Canada, 80 μg STX/100g edible part). The toxicity of bivalves, such as Japanese scallop, containing mainly GTXs could be reduced below the quarantine limit by heating under conditions deduced from the regression curves of GTXs (Table 1). Several unknown compounds were formed when PSP was heated above 100°C. Their structures as well as properties remain to be examined.

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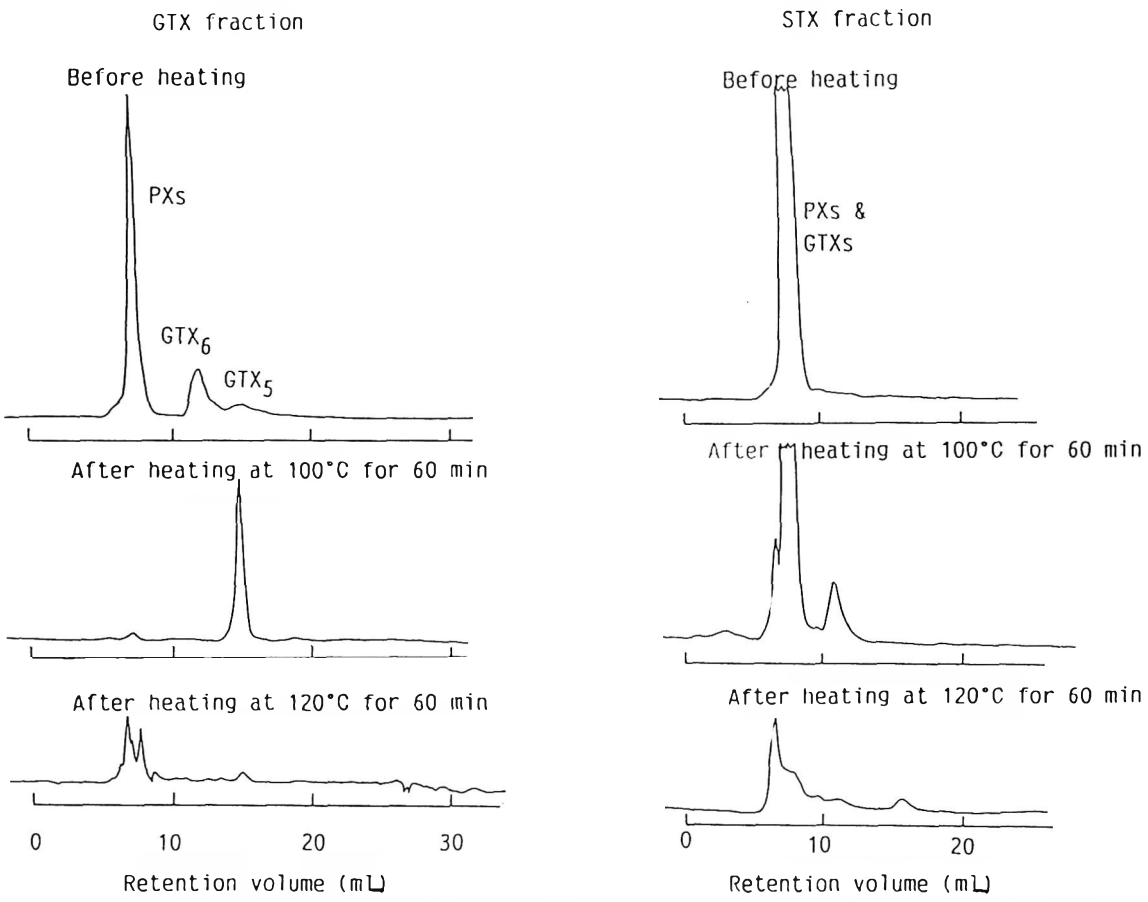

Fig. 5—HPLC of GTX and STX fractions from a toxic oyster homogenate, before and after heating at 100 and 120°C for 60 min.

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Influence of Maturity on the Volatile Aroma Compounds from Fresh Pacific and Great Lakes Salmon

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ABSTRACT

Volatile aroma compounds from freshly harvested prime and spawning-condition salmon (*Oncorhynchus sp.*) from the Pacific Ocean (chinook, sockeye, chum, coho and pink) and the Great Lakes (chinook, coho and pink) were quantitatively measured. Both prime and spawning-condition salmon had 1-octen-3-one, 1,5-octadien-3-one, 1-octen-3-ol, 1,5-octadien-3-ol, 2-octen-1-ol, and 2,5-octadien-1-ol which contributed distinct and characteristic plant-like aromas to the fish. More pronounced aromas of spawning-conditions almon were attributed to greater concentrations of the 8-carbon compounds in combination with occurrence of (E)-2-nonenal, (E)-2,(Z)-6-nonadienal, 6-nonen-1-ol, and 3,6-nonadien-1-ol which added sweet, cucumber- or melon-like aroma notes. The 9-carbon compounds may have resulted from biochemical regulation of physiologically active lipid-derived substances which activate mucus secretion in salmon approaching sexual maturity.

Key Words: salmon, fish, volatile-aroma, maturity, biochemical-regulation

INTRODUCTION

Prime Pacific salmon (*Oncorhynchus spp.*) when prepared into hot-smoked, canned or freshly cooked products are highly prized because of their distinctive pink to orange flesh and rich flavor. Additionally, raw forms of salmon are widely consumed as sushi and cured, cold-smoked lox. The degree of orange pigmentation as well as the flavor and texture of prime fish varies among different species of salmon (Bolton et al., 1967; Schmidt and Cuthbert, 1969; Scott and Crossman, 1973; Rearden, 1983). Collectively these features serve as a basis for quality rating and corresponding consumer appeal (Bolton et al., 1967; Ostrander et al., 1967; Francis and Clydesdale, 1977; Browning, 1980; Josephson and Lindsay, 1987).

The transition of prime ocean-dwelling salmon into spawning-condition salmon occurs as the various species of adult fish approach brackish waters and rivers, and this maturation involves a cessation of feeding and a mobilization of muscle lipids and carotenoid pigments into the gonads and skin (Idler and Bitners, 1958; Ota and Yamada, 1974; Kitahara, 1984). Alternatively, Pacific salmon that have been adapted to the Great Lakes complete the life cycle entirely in a freshwater environment, and sexual maturation proceeds according to age and seasonal influences. The transition to spawning-condition fish in either case yields lower quality salmon. This is, in part, caused by the loss of richness of the flesh when water replaces the fat in the muscle tissue (Idler and Bitners, 1958; Hatano, 1983; Kitahara, 1984), and the carotenoids have been depleted. In addition, mature fish have thick skins with substantially enhanced coatings of slime, and the aroma of these fish differs from prime ocean-caught salmon.

Increased consumer interest in fish and other seafoods has created greater demands on quality-related features and freshness as well as means to objectively measure them. Earlier research (Josephson et al., 1983; Josephson and Lindsay, 1986) has demonstrated that characterizing aroma compounds for fresh fish are present in both the tissue and the slime, but higher concentrations occur in the slime. Therefore, the objective of our investigation was to quantitatively measure the characterizing volatile aroma compounds in the slime of five species of Pacific salmon to determine differences in aroma quality of prime and spawning-condition fish. It was a further purpose to investigate the volatile compounds of some of the species of salmon adapted to the Great Lakes to assess the effects of freshwater environments on the aroma quality of freshly caught fish.

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MATERIALS & METHODS

BRIGHT adult coho (*Oncorhynchus kisutch*), sockeye (*Oncorhynchus nerka*), pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon (average weights 4, 3, 1.5, and 4 kg, respectively) were commercially harvested in July from saltwater off the mouth of the Kenai River (Alaska). These fish were held on ice during shipment and analyzed within 3 days after capture. Sexually-mature Pacific coho and sockeye salmon (average weights 4 and 3 kg, respectively) were sport-caught 110 and 125 km, respectively, upstream from the mouth of the Susitna River (Alaska). Fish were iced during shipment and analyzed 3 to 4 days after capture. Sexually mature Pacific chinook salmon (*Oncorhynchus tshawytscha* average weight 13 kg) which had been in freshwater environments for about 30 days, were obtained as ice-glazed fish from the Petersburg (Alaska) area. Fish held frozen for short storage times (< 5 days) provided very similar profiles of volatiles as found in iced fish. These chinook salmon had been harvested from a hatchery 2 days prior to receiving. Sexually mature pink and chum salmon (average weights 1.5 and 3 kg, respectively) were harvested in September from the San Juan Hatchery (Sawmill Bay area; Cordova, Alaska) which is located adjacent to the Prince William Sound (Alaska). These salmon were iced and analyzed 3 days after harvest. All salmon were whole and noneviscerated before analysis, and segregation of males and females was not attempted because of limited samples.

Adult bright coho and chinook salmon that had spent their entire life cycle in freshwater were obtained from Lake Michigan in August prior to spawning (average weights 3 and 9 kg, respectively; sport harvested, Sheboygan, WI area) and in October during spawning (average weights 3 and 10 kg, respectively; electroshocked, Sheboygan River, Kohler, WI). These fish were iced immediately after capture and maintained in that condition until analyzed within 12 hr. Sexually mature pink salmon were obtained in September (average weight 1 kg) from the French River, a tributary of Lake Superior (Minnesota). Before analysis these fish were held for 2 days on ice after having been harvested from a weir.

Analysis of volatile compounds

Extracts from salmon were prepared by immersing a single whole fish in 400 mL saturated NaCl solution contained in a large polyethylene bag, followed by agitating to recover most of the slime layer as an extract. An internal standard consisting of 400 μL of a solution of ethyl heptanoate (18.4 μg) in ethyl ether was next added to each extract. Headspace volatiles were collected from extracts by purging with a stream of nitrogen (150 mL/min for 2.5 hr) through each extract at room temperature (21°C) onto Tenax GC as described by Olafsdottir et al. (1985). Viscous slime extracts from sexually mature salmon foamed excessively, and these samples were extracted by directing a stream of nitrogen onto the surface of the continuously-stirred liquids rather than through liquids to purge volatiles onto Tenax GC traps. The aromas of all slime-extract samples were greatly diminished after

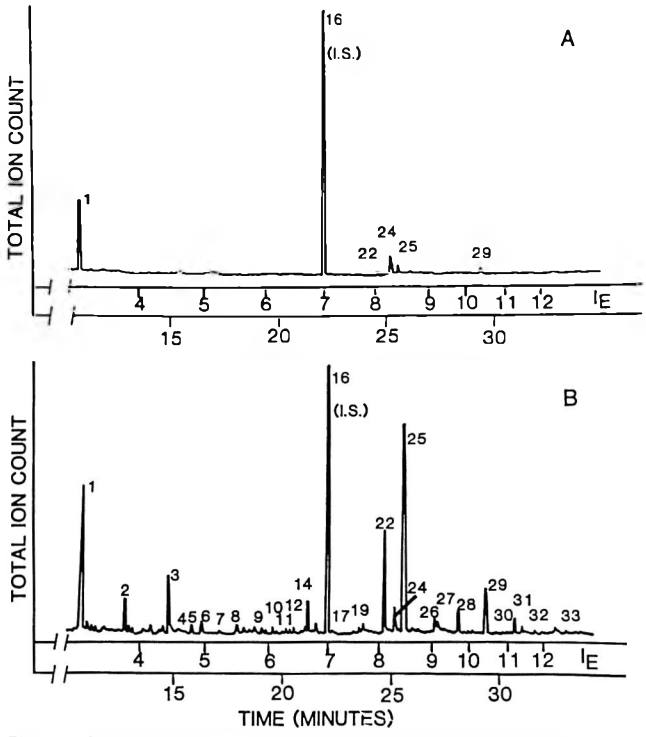

Fig. 1—Capillary column gas chromatograms of volatile aroma compounds from (A) prime, saltwater and (B) spawning-condition, freshwater Pacific Ocean salmon. Peak numbers correspond to compounds listed in Table 1.

purging for 2.5 hr indicating that volatile aroma compounds had been transferred onto the Tenax GC.

Volatile compounds in ethyl ether extracts from Tenax GC traps were quantified after separation with a Varian 1740 gas chromatograph (Varian Associates, Palo Alto, CA) equipped with a packed column, an effluent splitter, and a flame ionization detector (FID). The configuration of the effluent splitter allowed simultaneous FID recordings and odor evaluations of eluting compounds. A 3 m × 2 mm i.d. silane-deactivated glass column packed with 7% Carbowax 20 M on 80-100 mesh Chromosorb W AW/DMCS and programmed from 50° to 200°C/min was used. Flow rates were: nitrogen carrier gas 24, hydrogen 24, and air flow 240 mL/min. Injection port was 250°C and detector 235°C.

Capillary column GC, in conjunction with mass spectrometric analyses for identification of volatiles in ethyl ether extracts from Tenax GC traps, was performed as described by Josephson et al. (1983). A Supelcowax 10 (60 m × .25 mm i.d.) fused silica capillary column (Supelco Inc., Bellefonte, PA) operated with helium carrier gas was used. After injection, the temperature was held at 50°C for 5 min, programmed at 6°C/min to 140°C and followed by a rate of 10°C/min from 140°C to 220°C.

Identifications of compounds were based on computer matchings of full or partial mass spectra of compounds published in *EPA/NIH Mass Spectral Data Base* (Heller and Milne, 1975, 1980), and by coincidence of mass spectral patterns from isolated compounds with those of authentic compounds as well as coincidence for retention indices (I_E, Van den Dool and Kratz, 1963). The aromas of fish and gc column effluents were assessed by the authors.

RESULTS & DISCUSSION

EACH of the five *Oncorhynchus* species of salmon gave qualitatively similar profiles of volatile compounds. The capillary column gas chromatograms shown for coho in Fig. 1 illustrate differences in volatiles between prime and spawning-condition salmon. The identities of peaks in the chromatograms are presented in Table 1 along with quantitative data for individual compounds.

Prime quality salmon from saltwater exhibited substantially lower aroma intensities and concentrations of volatile aroma compounds (Fig. 1A; Table 1) compared to corresponding

Table 1—Volatile aroma compounds identified in Pacific coho salmon obtained from (A) prime, saltwater and (B) spawning-condition, freshwater Pacific Ocean salmon

Peak no.[a]	Compound	Concentration in salmon extract: Prime (Saltwater) (ppb)[c]	Concentration in salmon extract: Spawning-condition (Freshwater) (ppb)[c]	I_E[b]
1.	ethanol	19.5	24.8	3.00
2.	2-butanol	–[d]	6.5	3.78
3.	hexanal	–	8.3	4.49
	1,3,5-octatriene	–	0.3	4.64
4.	1,3,5-octatriene	–	0.3	4.77
5.	2-heptanone	–	0.5	4.85
6.	2-pentenal	–	1.0	4.97
7.	1-penten-3-ol	–	0.5	5.24
8.	heptanal	–	0.8	5.46
9.	2-hexenal	–	0.5	5.81
10.	1-pentanol	–	0.3	6.05
11.	(Z)-4-heptenal	–	0.3	6.08
12.	3-octanone	–	0.3	6.25
13.	octanal	–	0.3	6.52
14.	1-octen-3-one	trace	0.5	6.64
15.	cyclopentanol	–	0.5	6.82
16.	ethyl heptanoate (Internal Standard)	45	45	7.00
17.	1-hexanol	–	0.3	7.12
18.	1,5-octadien-3-one	trace	trace	7.36
	3-hexen-1-ol	–	0.3	7.46
19.	3-octanol	–	1.0	7.54
20.	2-nonanone	–	0.3	7.64
21.	nonal	–	0.3	7.68
22.	1-octen-3-ol	1.0	18.8	8.07
23.	1-heptanol	–	0.3	8.13
24.	acetic acid	–	3.3	8.27
25.	1,5-octadien-3-ol	3.3	37.1	8.41
26.	(E)-2-nonenal	–	trace	9.00
27.	(E)-2,(2)-6-nonadienal	–	0.3	9.46
	2-undecanone	–	0.3	9.46
28.	2-octen-1-ol	–	3.5	9.72
	1-nonanol	–	0.3	10.02
29.	2,5-octadien-1-ol	0.1	7.8	10.36
30.	6-nonen-1-ol	–	trace	10.42
31.	3,6-nonadien-1-ol	–	0.3	11.07
32.	2,4-decadienal	–	0.3	11.62
33.	unknown	–	0.3	12.40

[a] Peak no. correspond to those shown in Fig. 1.
[b] Retention Indices on Carbowax 20M (I_E; Van den Dool and Kratz, 1964).
[c] Average of duplicate analyses; ranges within ± 20% of mean.
[d] Not detected.

spawning-condition salmon residing in freshwater (Fig. 1B; Table 1). Volatile compounds identified in salmon direct from saltwater included the 8-carbon alcohols, 1-octen-3-ol, 1,5-octadien-3-ol, and 2,5-octadien-1-ol which impart characterizing green, plant-like aromas (Josephson and Lindsay, 1986). Only trace amounts of 1-octen-3-one and 1,5-octadien-3-one were detected by their characteristic aromas (Pyysalo and Suihko, 1976; Swoboda and Peers, 1977) in extracts from saltwater fish as they eluted during packed column GC separations.

Spawning-condition ocean coho salmon obtained from freshwater (Fig. 1B; Table 1) possessed substantially higher concentrations of the 8-carbon volatile alcohols and ketones than those captured from saltwater. This seemed to indicate that higher lipoxygenase activity (German and Kinsella, 1985; Josephson et al., 1987) occurred in the spawning-condition fish. The 9-carbon compounds, 2-nonenal, 2,6-nonadienal, 6-nonen-1-ol, 3,6-nonadien-1-ol, and an unidentified compound (I_E = 12.40) all contributed green, cucumber-like aroma notes to the spawning-condition coho salmon. Similar profiles of 8-carbon and 9-carbon volatile compounds have been reported for various freshwater fish (Josephson et al., 1983, 1984; Josephson and Lindsay, 1986). Each group of compounds provided distinct characterizing aromas to the different fish species. The occurrence of the 8-carbon compounds in salmon extends hypotheses that these compounds appear to be present in all species of fish (Josephson et al., 1984; Josephson and Lindsay, 1986).

Table 2—Concentrations of influential lipid-derived volatile aroma compounds from various freshly-harvested Pacific Ocean salmon

Samples	Concentrations of volatile compounds											
	Hexanal	1-Octen-3-one	1,5-Octadien-3-one	1-Octen-3-ol	1,5-Octadien-3-ol	2-Octen-1-ol	2,5-Octadien-1-ol	2-Nonenal	2,6-Nonadienal	6-Nonen-1-ol	3,6-Nonadien-1-ol	Unknown
	(ppb[b])											
Prime fish (saltwater)												
Pink[c]	—[d]	tr	tr	0.5	1.3	—	0.2	—	—	—	—	—
Sockeye[c]	—	tr	tr	0.7	1.4	—	0.2	—	—	—	—	—
Chum[c]	—	tr	tr	1.0	3.3	—	0.8	—	—	—	—	—
Coho[c]	—	tr	tr	0.4	1.1	—	0.1	—	—	—	—	—
Chinook	Not available											
Spawning-condition fish (freshwater)												
Pink[f]	1.3	tr	tr	4.3	5.8	1.0	1.6	1.0	1.5	—	0.3	—
Sockeye[g]	5.8	0.3	tr	15.8	31.8	4.3	8.0	tr	0.1	0.2	0.3	0.3
Chum[f]	1.3	tr	tr	26.8	43.1	7.5	18.0	2.3	tr		2.5	1.8
Coho[g]	8.3	0.5	tr	18.8	37.0	3.5	7.8	tr	0.3	tr	0.3	0.3
Chinook[h]	9.5	3.0	tr	38.4	94.8	11.3	24.3	tr	1.8	0.5	1.0	2.5

[a] Cucumber-like odor quality-m/z: 71(100) 43(81) 83(49) 98(46) 56(45) 89(30) 143(10); I_E = 12.40; Carbowax 20M.
[b] Concentration in 400 mL extract from a single fish.
[c] Cook Inlet; Kenai River area- saltwater- July.
[d] Not detected.
[e] tr = trace (based on odor assessment).
[f] Prince William Sound area, San Juan Hatchery, Cordova, AK.
[g] Susitna River tributary—approx. 75 km upstream from Cook Inlet.
[h] Southeast Alaska—Petersburg area—local freshwater river.

Table 3—Concentrations of influential lipid-derived volatile aroma compounds from various species of freshly-harvested Great Lakes salmon

Samples	Concentrations of volatile compounds											
	Hexanal	1-Octen-3-one	1,5-Octadien-3-one	1-Octen-3-ol	1,5-Octadien-3-ol	2-Octen-1-ol	2,5-Octadien-1-ol	2-Nonenal	2,6-Nonadienal	6-Nonen-1-ol	3,6-Nonadien-1-ol	Unknown
	(ppb[b])											
Prime fish (Freshwater)												
Coho[c]	0.52	tr[d]	tr	8.0	2.5	3.3	1.0	—[e]	—	—	—	—
Chinook[c]	0.8	tr	tr	6.3	1.8	2.0	1.8	tr	tr	—	—	—
Spawning-condition fish (freshwater)												
Coho[f]	tr	tr	0.03	5.8	1.6	1.5	0.5	0.16	0.08	tr	tr	0.5
Chinook[f]	9.5	tr	0.05	10.3	2.2	2.5	1.0	0.40	0.28	—	0.05	0.3
Pink[g]	4.0	0.13	tr	2.8	1.0	0.8	0.6	tr	0.1	—	tr	0.2

[a] Cucumber-like odor quality- m/z: 71(100) 43(81) 83(49) 98(46) 56(45) 89(30) 143(10); I_E = 12.40; Carbowax 20M.
[b] Concentration in 400 mL extract from a single fish.
[c] Lake Michigan—5 km offshore—August.
[d] tr = trace (based on odor assessment).
[e] Not detected.
[f] Lake Michigan tributary—Sheboygan River—8 km upstream—Lake September.
[g] Lake Superior tributary—2 km upstream from the mouth of the French River—Early September.

Data for concentrations of volatiles from the survey of five species of salmon obtained from Pacific ocean, and chinook, coho and pink salmon from the Greak Lakes are summarized in Tables 2 and 3. Although the five species of salmon had qualitatively similar profiles of aroma compounds during comparable life cycle stages, varying physiological requirements appeared to alter the kind and amount of volatiles present. Chinook salmon residing in freshwater were especially characterized by course, plant-like aromas that were caused by elevated levels of both hexanal and 8-carbon volatile compounds which were more abundant in chinook than in any other salmon (Tables 2 and 3). In these mature chinook, the intense aroma of hexanal and 8-carbon volatiles largely masked the cucumber-, melon-like aromas of the 9-carbon volatile compounds. On the other hand, spawning-condition chum salmon had distinct, melon-like aromas when obtained from freshwater sites. Chum salmon had the greatest concentrations of 9-carbon volatile compounds along with the lowest concentrations of hexanal (Table 2) observed for any salmon species.

Some hexanal was found in adult pre-spawning Greak Lakes salmon samples (Table 3), but it was absent from the pre-spawning-condition Pacific Ocean salmon obtained from saltwater environments (Table 2). However, spawning-condition salmon from freshwater for both the Pacific Ocean (Table 2) and Lake Michigan (Table 3) stocks contained hexanal in the profile of volatile compounds. These observations suggested that the production of hexanal might be associated with regulation of physiological processes that are invoked by freshwater environments.

Generally, the amounts of the 8-carbon volatile aroma compounds relative to the sum of the volatile compounds were quite consistent among species of salmon when environmental and maturity factors were rationalized (Tables 2 and 3). However, viewing absolute amounts of compounds, adult coho and chinook salmon from the Great Lakes (Table 3) had substantially lower amounts of 1,5-octadien-3-ol and 2,5-octadien-1-ol relative to the Pacific Ocean salmon surveyed. Both of these volatiles are derived from enzymic conversions of n-3 polyunsaturated fatty acids (Josephson and Lindsay, 1986), and their abundance may reflect some increased essential biochem-

ical regulatory role for n-3 fatty acids in pre-spawning adult salmon residing in freshwater.

The 9-carbon volatile carbonyls and alcohols were not present in pre-spawning salmon from either the Pacific Ocean or Lake Michigan stocks (Table 2 and 3), but were found in notable concentrations in the extracts of all spawning-condition salmon (Tables 2 and 3). 2-Nonenal and 2,6-nonadienal can be formed via both enzyme-mediated and non-enzymic pathways, but 6-nonen-1-ol and 3,6-nonadien-1-ol can be formed only via enzyme-mediated reactions (Kemp et al., 1974; Kemp 1975; Hatanaka et al., 1975). The trace occurrences for 2-nonenal and 2,6-nonadienal in prime, pre-spawning chinook salmon from the Great Lakes (Table 3) were interpreted as inconsequential amounts arising from nonenzymic degradations. When held refrigerated up to 7 days, ocean salmon also accumulated low concentrations of 2-nonenal and 2,6-nonadienal through fatty acid autoxidation (data not shown). However, significant accumulations of autoxidatively-formed 9-carbon aldehydes are always accompanied by similarly-formed 2,4-alkadienals (C7 and C10) which eventually result in a masking of the green-notes to yield oxidized fish aromas (McGill et al., 1974, 1977; Swoboda and Peers, 1977).

Because 2-nonenal, 2,6-nonadienal, 6-nonen-1-ol and 3,6-nonadien-1-ol have also been found in freshwater lake whitefish (*Coregonus clupeaformis*), smelt (*Osmerus mordax*), muskellunge (*Esox masquinongy*), northern pike (*Esox lucius*), ciscoe (*Coregonus artedii*) (Josephson et al., 1983, 1984) and Pacific oysters (*Crassostrea gigas*; Josephson et al., 1985) regardless of season, their biosynthesis appears to be related to some physiological process other than reproductive state or function. Salmon enroute to spawning require means to enhance physical protection, including skin thickening and mucus secretion among other physiologically-vital systems. This may be related to the generation of 9-carbon compounds. We hypothesize regulatory roles involving the formation and inactivation of physiologically-active lipids such as leukotrienes which activate mucus secretion and related systems, are involved in formation of the 9-carbon fresh fish volatiles in fish and molluscs (Josephson and Lindsay, 1986).

For salmon, apparently, less osmoregulation demands would be imposed on the skin barrier when they are in saltwater environments than when in freshwater, and slime exists only to a limited extent on saltwater fish compared to freshwater fish. The greater amounts of slime observed on salmon residing in freshwater environments, thus, indicates that the rate of mucus or slime production has been stimulated in those fish. Leukotrienes C_4 and D_4 stimulate or modulate mucus secretion in mammals (Goetzl et al., 1983; Johnson et al., 1983; Richardson et al., 1983) where they are derived from arachidonic acid (C20:4, n-6,) and similar systems may occur in fish.

Another potential role for leukotriene synthesis could relate to the vulnerability of the fish to injury during spawning migrations. Following tissue damage leukotriene B_4 (LTB_4) is produced, and this compound has been shown to exert powerful chemotactic influences *in vitro* on leukocytes (Ford-Hutchinson et al., 1980; Goetzl and Pickett, 1980; Brian et al., 1984. Hunt and Rowley (1986) have demonstrated that LTB_4 enhances the migration of dogfish leukocytes *in vitro*, and this is the first known report of the function of LTB_4 in a nonmammalian vertebrate. Investigations of leukotrienes in fish skin locations have not been reported, but Piomelli (1985) has isolated leukotrienes C_4, D_4 and E_4 from the gill tissues of the American eel which is a teleost fish.

German et al. (1986) and German and Kinsella (1985, 1986) have isolated a 12-lipoxygenase from both the gill tissue and skin of rainbow trout (*Salmon gairdneri*). The enzyme may serve a role in the defensive systems located at the skin-water interface via initiation of the biogenesis of physiologically-active compounds. 12-Lipoxygenase converts arachidonic acid to 12-hydroperoxy-5, 8, 10, 14-eicosatetraeonic acid (12-HPETE), and Maclouf et al. (1983) have demonstrated a role for 12-HPETE in activating leukotriene biosynthesis in leukocytes. Further research into the validity of this hypothetical biological control mechanism appears warranted because it could provide insights into lipid nutrition of aquacultured fish.

The formation of high levels of (E)-2,(Z)-6-spawning-condition salmon provides the required precursor for the formation of stale-flavored aldehyde, (Z)-4-heptenal, via water-mediated retro-aldol condensation reactions (Josephson and Lindsay, 1987). McGill et al. (1974, 1977) have reported the accumulation of (Z)-4-heptenal in frozen stored cod to an extent that the quality of cod was greatly impaired (Ross and Love, 1979). Concentrations of (Z)-4-heptenal in spawning-condition salmon increased during refrigerated storage through 14 days (data not shown), and the stale aromas that developed in stored salmon appeared to reflect the presence of (Z)-4-heptenal. Accelerated rates of (Z)-4-heptenal formation from (E)-2, (Z)-6-nonadienal can occur during heating (Josephson and Lindsay, 1987). Therefore, spawning condition salmon would be expected to be more susceptible to development of storage or stale flavors than prime condition salmon which lack (E)-2, (Z)-6-nonadienal in their volatile compound profile.

In summary, salmon from both the Pacific Ocean and Great Lakes had 8-carbon volatile alcohols and ketones regardless of stage of maturity. Spawning-condition salmon possessed abundant concentrations of 9-carbon volatile alcohols and aldehydes which were absent in pre-spawning-condition salmon from either saltwater (Pacific Ocean) or freshwater (Great Lakes). Hexanal was found in all salmon residing in freshwater, but not in those residing in saltwater. Overall, high quality salmon harvested from saltwater had mild fresh plant-like aromas and flavors, and prime all-freshwater salmon had more pronounced plant-like aromas than high quality saltwater salmon. Further research is needed to more clearly define the role of the volatile carbonyls and alcohols along with volatile compounds from feed and environmental sources in determining the quality of salmon flavors.

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Model for Fresh Produce Respiration in Modified Atmospheres Based on Principles of Enzyme Kinetics

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ABSTRACT

A respiration model, based on enzyme kinetics, was proposed for predicting respiration rates of fresh produce as a function of O_2 and CO_2 concentrations. In this model, the dependence of respiration on O_2 was assumed to follow a Michaelis-Menten type equation ($r = V_m[O_2]/\{K_m + [O_2]\}$), and the effect of CO_2 on respiration to follow an uncompetitive inhibition model ($r = V_m[O_2]/\{K_m + (1 + [CO_2]/K_i)\ [O_2]\}$). The model predictions agreed well with published data for a variety of commodities and with experimental data for cut broccoli. Fresh produce respiration rates (O_2 consumption or CO_2 evolution) at various O_2 and CO_2 concentrations, as well as transient and equilibrium gas concentrations within permeable packages, could be accurately predicted with the model equations.

Key Words: fresh-produce, respiration, modelling, modified-atmosphere, enzyme kinetics

INTRODUCTION

MODIFIED ATMOSPHERE packaging (MAP) is a technique used to extend the shelf life of fresh produce inside a permeable package. MAP reduces the rates of respiration and ethylene production, retards softening and changes occurring in the produce, by creating and maintaining an optimum microatmosphere (usually reduced O_2 and elevated CO_2 levels) inside the package. A desired microatmosphere may be established rapidly by flushing the headspace of the package with a desired gas mixture, and the atmosphere maintained by controlling the influx of O_2 and efflux of CO_2 with a properly selected permeable film. The desired microatmosphere could also be established, at a slow rate, by allowing the produce to respire inside the package to attain an equilibrium atmosphere. In either case, the dynamics of the microatmosphere depends on the produce (weight and respiration rate), the package (thickness and gas permeabilities of the plastic film, free volume, and surface area of the package) and the environment (ambient gas compositions and storage temperature).

Since the early 1960's, several attempts have been made to model the dynamics of the microatmosphere in MAP systems. Jurin and Karel (1963) developed a graphical method for determining the equilibrium O_2 and CO_2 concentrations inside a permeable package. Veeraju and Karel (1966) devised an analytical method using 2 plastic films with different permeabilities to control the concentrations of O_2 and CO_2 independently. Henig and Gilbert (1975) derived two simultaneous first-order differential O_2 and CO_2 balance equations, solved them numerically to simulate the transient gas concentrations in model packages, and analyzed the effect of various package variables on gas composition with time. Hayakawa et al. (1975) modified the equations of Henig and Gilbert (1975), and obtained analytical solutions for the model package parameters. All of the mentioned methods required experimental respiration rates as a function of O_2 and CO_2 concentrations.

Unfortunately, there is still a paucity of published respiration data in modified atmospheres because of the difficult and time-consuming experiments necessary for measuring respiration rates, as well as the complex nature of the respiration process that impedes development of predictive models. Recently, some attempts were made to correlate respiration rates with in-package O_2 and CO_2 concentrations. Yang and Chinnan (1988) used a quadratic function with 9 parameters to correlate respiration rates of tomato with O_2 and CO_2 concentrations and storage time, and used another quadratic function with three parameters to correlate the respiration rate with storage time. Cameron et al. (1989) used a trial-and-error approach to curve fit O_2 consumption of tomato with an exponential type function which consisted of four coefficients. These empirical approaches, however, were somewhat arbitrary and limited in usefulness.

Our work was aimed at developing and verifying a respiration model, based on the underlying principles of enzyme kinetics, for predicting respiration rates of fresh produce as a function of both O_2 and CO_2 concentrations. The soundness of the model was evaluated with published respiration rate data, as well as with experimental data.

RESPIRATION MODEL

THE PROCESS of respiration in fruits and vegetables may be represented by the following chemical reaction (Ryall and Pentzer, 1979; 1982):

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + \text{Energy} \quad (1)$$

Aerobic respiration involves a series of enzymatic reactions that take place through the metabolic pathways of glycolysis, the tricarboxylic acid (TCA) cycle, and the associated electron transport system (Kader, 1987). The respiration rate is also governed by diffusion of O_2 and CO_2 through plant tissues. The respiratory quotient (R.Q.), the ratio of CO_2 produced to O_2 consumed, ranges from 0.7 to 1.3 depending upon the metabolic substrate (Kader, 1987; Kader et al., 1989), and is affected by modified atmosphere conditions (i.e. reduced O_2 and elevated CO_2). From these observations, it is obvious that the respiration of produce is a complicated and difficult process to model. If all factors involved in enzymatic reactions were considered in a respiration model, it would become so complex that it would be of limited value. Recognizing this, we attempted to find a simple model to describe the respiration rate as a function of O_2 and CO_2 concentrations.

Recently, Yang and Chinnan (1988) suggested that the principles of enzyme kinetics might be appropriate for modeling respiration of produce. We speculated that a Michaelis-Menten type equation was useful based on the following rationale. Respiration in fresh produce is possibly governed by an enzymatic reaction catalyzed by an allosteric enzyme(s) and regulated through feedback inhibition (Solomos, 1983). The solubility and diffusion of O_2 and CO_2 in the plant tissue possibly limit the rate of respiration. A similarity between fresh produce respiration and microbial respiration can be shown by

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examining the published data on respiration. Therefore, it was reasonable to speculate that the Michaelis-Menten equation may describe the respiration of produce, because that equation has commonly been used to describe enzyme reactions even in unpurified state, when certain assumptions were applied (Bailey and Ollis, 1977). The Michaelis-Menten equation has also been used to describe the respiration rate of microorganisms in industrial aerobic fermentation processes (Arnold and Steel, 1958; Stanbury and Whitaker, 1984) and diffusion controlled reactions (Bailey and Ollis, 1977).

Based on the similarity of fresh produce respiration and microbial respiration, Michaelis-Menten type enzyme kinetics was applied to model respiration of fresh produce. When CO_2 is absent, the dependence of respiration on O_2 concentration can be described with

$$r = \frac{V_m [O_2]}{K_m + [O_2]} \quad (2)$$

The dependence of respiration on CO_2 concentration was modeled based on the uncompetitive inhibition, which has been used exclusively for product inhibition in reactions involving several substrates and products (Cornish-Bowden, 1979). For the respiration process, the uncompetitive inhibition mechanism due to CO_2 is expressed as

$$r = \frac{V_m [O_2]}{K_m + (1 + [CO_2]/K_i) [O_2]} \quad (3)$$

Eq. (3) is assumed to be valid as long as the produce undergoes aerobic respiration (i.e. sufficient O_2 is available to act as substrate).

MATERIALS & METHODS

THE MODEL was first tested using published respiration data. Since certain necessary information (such as package size and film permeability) was not reported in the references used to obtain the data, we also conducted experiments using cut broccoli to further test the model.

Sample preparation

California broccoli, purchased in case quantities from a supermarket in New Jersey, was stored at 0°C on ice prior to each experiment. Broccoli florets of uniform size (6.5 cm length, 4–5 cm width at top) were cut, rinsed in cold water for 20 sec, and spun in a salad spinner for 45 sec to remove excess water. The florets were equilibrated to the experimental temperature for 30 min before each experiment began. All experiments were conducted at 24 ± 0.5°C. Three sets of experiments were conducted, each set using a different lot of broccoli.

Respiration rate as function of O_2

The first set of experiments measured the respiration rates of cut broccoli as a function of O_2 concentration, in the absence of CO_2, using an open or flow-through system (Lee, 1987). Five O_2 concentrations were tested (1.7, 3.3, 6.2, 10.1, and 19.4%). These O_2 concentrations were measured at the outlet, since we found that the gas surrounding the produce in the respiration chamber had the same composition as that of the outlet gas. The small amount of CO_2 accumulation in the respiration chamber was considered negligible in these experiments.

The gas mixing system was a variation of that used by Fidler and North (1967). O_2 and N_2 were supplied from cylinders with flow rate of each gas controlled ± 0.5 mL/min, using an appropriate length of glass capillary tubing 0.2 or 0.3 mm diameter (Andrew's Glass Co., Vineland, NJ) and a water column of predetermined height to supply constant pressure. To facilitate mixing, the two gases were combined in a sealed 250 mL flask equipped with inlet and outlet fittings and a silicone gas sampling port prior to entering the respiration chambers.

The respiration chambers were constructed using 3.8 L glass jars with metal caps equipped with brass fittings and Tygon® tubings. Each jar contained 20 pieces of broccoli weighing about 185g. The gas mixture was passed through the jars at an average 55 mL/min, and the outlets of the jars were connected to sealed 250 mL flasks where the outlet gas was sampled. The experiment began after the respiration chambers were adequately flushed with the test gas mixture for about 60 min. Respiration rates were determined from the difference between inlet and outlet gas concentrations after correcting for humidity, which was necessary because the inlet gas was essentially dry but the outlet gas was humidified by the H_2O produced in the respiration process. Carbon dioxide production and O_2 uptake were measured using a Hewlett Packard 5890A gas chromatograph equipped with a thermal conductivity detector. An Alltech CTR I column (Alltech Associates, Inc. Deerfield, IL) was used with helium as a carrier gas at 65 mL/min and the column at 30°C. For each O_2 level, three measurements were made on each of the two jars, and the average of these six readings was used for data analysis.

Respiration as function of CO_2

The second set of experiments used the open system to measure the respiration rates as a function of CO_2 concentration. Six levels of CO_2 were tested at each of two constant inlet O_2 levels (0.9, 4.0, 7.3, 11.1, 14.5, and 17.8% CO_2 at 12.5% O_2; 1.1, 4.0, 7.1, 10.5, 13.8, and 16.5% CO_2 at 21.0% O_2). These CO_2 levels represented outlet gas concentration. Inlet gas composition and flow rate (average 47 mL/min) were controlled as before, and broccoli weight was about 185g. Respiration chambers were constructed using 1L jars. Each O_2 level was tested on two consecutive days, and three readings were taken for each jar on both days, for a total of six readings. The six measurements were averaged for data analysis.

Package simulation

The purpose of the third set of experiments was to verify that the respiration model could predict the respiration rates inside a model permeable package. First, the model parameters were estimated from experimental data. The open system method was used to measure the respiration rates of cut broccoli as a function of 12 different O_2/CO_2 combinations (3.4/0.9, 3.4/4.6, 3.5/7.7, 2.6/10.3, 8.9/1.1, 9.6/4.4, 9.6/7.9, 9.4/12.1, 16.0/1.0, 16.5/4.1, 16.2/7.3, 16.5/11.2). Gas composition and flow rate (average 45 mL/min) were controlled as described before, and 1L jars were used for respiration chambers. Two readings were taken for each gas combination, and the average of the 2 measurements was used to estimate the values of the model parameters through regression analysis.

Second, the respiration rates inside a model permeable package was measured with time. The package, initially containing air, was constructed of 2 mil low density polyethylene film (Dow Chemical Co., Midland, MI) and contained 137.3g of broccoli. The free volume inside the package was measured by injecting 4 mL methane gas into the package and measuring the resultant dilution after a 15 min equilibration period. O_2 and CO_2 permeabilities of the film at 24°C were measured using the method of Karel et al. (1963). The model package was equipped with a silicone sampling port, and headspace were sampled at about 30 min intervals and injected into the gas chromatograph for analysis. Sampling continued until the O_2 level inside the package reached 2%.

RESULTS & DISCUSSION

Confirmation using published data

The soundness of the respiration model was evaluated with published data. To examine the effect of O_2, respiration rate data as a function of O_2 in the presence of little or no CO_2 were collected and analyzed. Eq. (2) was linearized as Eq. (4) for the purpose of fitting the data: the resulting plot, $[O_2]/r$ versus $[O_2]$, referred to as a Hanes plot (Cornish-Bowden, 1979), was preferable to a Lineweaver-Burk plot because it yielded a more even distribution of error.

$$\frac{[O_2]}{r} = \frac{K_m}{V_m} + \frac{[O_2]}{V_m} \quad (4)$$

In general, the published data showed high linearity (Fig. 1), and the coefficients of determination (R^2) were above 0.95 (Table 1). The data of Henig and Gilbert (1975), however, did not show as strong linear relationship, probably because those researchers used an oversimplified assumption in generating their data (Kader et al., 1989). If the data of Henig and Gilbert

Table 1—Linearity of published data based on Eq. (4).

Respiration rate Expression	Commodity	Conditions of measurement	Coefficient of determination	Reference
CO_2 evolution (mg/kg·hr)	Broccoli	75°F (23.9°C) flushed system	0.988	Lieberman & Hardenburg (1954)[a]
O_2 consumption (mL/kg·hr)	Apple	20°C closed system, $[CO_2]<4\%$	0.957	Jurin & Karel (1963)
O_2 consumption (mL/kg·hr)	Banana	19°C closed system, $[CO_2]<3\%$	0.958	Karel and Go (1964)
O_2 consumption (mL/kg·hr)	Apple (Cox's Orange Pippin)	38°F (3.3°C) open system	0.997	Fidler & North (1967)
CO_2 evolution (mL/kg·hr)	Apple (Cox's Orange Pippin)	38°F (3.3°C) open system	0.999	Fidler & North (1967)
O_2 consumption (mL/kg·hr)	Tomato	23°C closed system, CO_2 absorbed	0.838	Henig & Gilbert (1975)
O_2 consumption (mL/kg·hr)	Tomato (pink)	25°C closed system, CO_2 absorbed	0.998	Cameron et al. (1989)

[a] Average of published data.

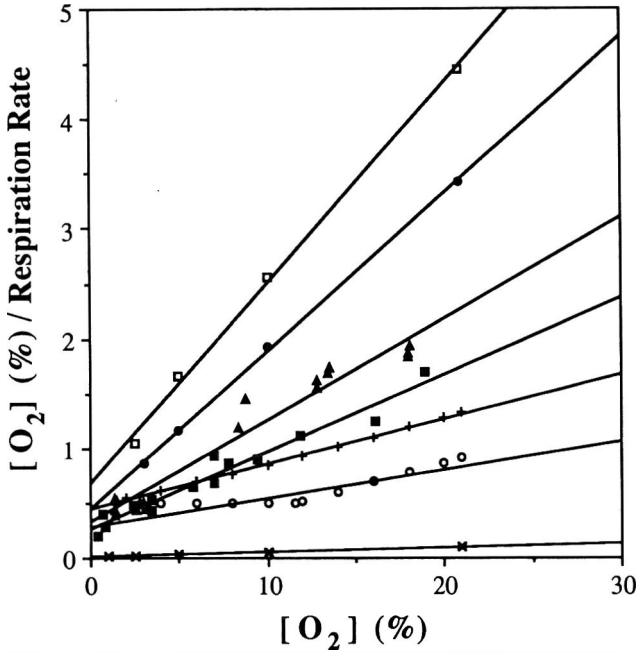

Fig. 1.—Hanes plot of published data on respiration as a function of O_2 concentration based on a fit by Eq. (4). For units, commodites and conditions of measurement, see Table 1. Respiration values in terms of O_2 consumption rate: ▲, Jurin and Karel (1963); ■, Karel and Go (1964); □, Fidler and North (1967); ○, Henig and Gilbert (1975); +, Cameron et al. (1989). Respiration values in terms of CO_2 evolution rate: ×, Lieberman and Hardenburg (1954); ●, Fidler and North (1967).

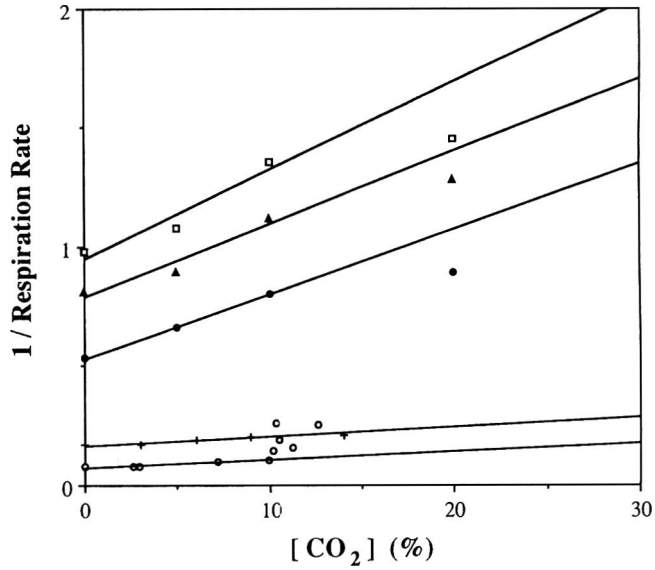

Fig. 2.—Published respiration data as a function of CO_2 concentration based on a fit by Eq. (5). For units, commodities and conditions of measurement, see Table 2. Respiration data: +, [Thornton (1933); ○, Jurin and Karel (1963); ●, 21% O_2, Lebermann et al. (1968); ▲, 5% O_2, Lebermann et al. (1968); □, 2% O_2, Lebermann et al. (1968).]

(1975) are excluded, we can conclude that the dependence of respiration rate on O_2 concentration can be expressed very well with the Michaelis-Menten type equation.

To examine the effect of CO_2 on respiration, Eq. (3) was linearized as

$$\frac{1}{r} = \frac{1}{V_m} + \frac{K_m}{V_m}\frac{1}{[O_2]} + \frac{1}{K_i V_m}[CO_2] \qquad (5)$$

If the model was applicable, the reciprocal of the respiration rate would be linear with CO_2 concentration at constant O_2 concentration. Published data show fairly strong linearity (Fig. 2), and the coefficients of determination were above 0.92 (Table 2). Analyzing the data of Lebermann et al. (1968), in which three O_2 concentrations were tested, showed that the slopes of the linear portion of the curves were nearly equal for each O_2 concentration, which is characteristic of uncompetitive type inhibition (Cornish-Bowden, 1979). At high CO_2 concentrations the linear dependence was somewhat disturbed—this same trend has also been observed for respiration of microorganisms as a function of dissolved CO_2 concentration (Ho et al., 1987). When CO_2 concentration was high, the inverse of the respiration rate fell below the linear portion of the curve for broccoli and asparagus, and increased abruptly for apple. Above certain CO_2 tolerance limits of a commodity, a significant increase in anaerobic respiration occurs, which can irreversibly damage the tissue (Kader, 1987; Kader et al., 1989). During anaerobic respiration or under conditions of high CO_2 concentration, the R.Q. may change and the dependence of respiration rate on O_2 or CO_2 may be very different. The response of the respiration process to high concentrations of CO_2 depends on the tolerance limit of the commodity (Thornton, 1933; Young et al., 1962; Kader et al., 1989). For example, apple has a lower CO_2 tolerance limit than broccoli or asparagus. The irregularity of the respiration data at high CO_2 concentrations seemed to be, at least partially, due to those reasons, and the data suggest the Eq. (5) is valid only up to the CO_2 tolerance limit of the commodity.

So far, the proper linearities according to Eq. (5) have been confirmed with published data. Furthermore, the proposed model was tested for its ability to predict the respiration rate at any O_2 and CO_2 concentration and to predict the composition of the microatmosphere within a permeable package. Therefore, it was necessary to estimate the model parameters of Eq. (5), V_m, K_m, and K_i. The data of Jurin and Karel (1963) was chosen

Table 2—Linearity of published data based on Eq. (5)

Respiration rate expression	Commodity	Conditions of measurement	Coefficient of determination	Reference
O_2 consumption (mg/kg·hr)	Asparagus	25°C closed system, $[O_2]$: 20%	0.965	Thornton (1933)[a,d]
CO_2 evolution (mL/kg·hr)	Apple	20°C closed system, $[O_2]$: 16–17%	0.946	Jurin & Karel (1963)[b]
CO_2 evolution (mmole/kg·hr)	Broccoli	45°F (7.2°C) closed system		Lebermann et al., (1968)[c,d]
		$[O_2]$: 21%	.000	
		$[O_2]$: 5%	0.934	
		$[O_2]$: 2%	0.926	

[a] Regression over 0–9% CO_2.
[b] Regression over 0–10% CO_2.
[c] Regression excludes 20% CO_2 values.
[d] Average of published data.

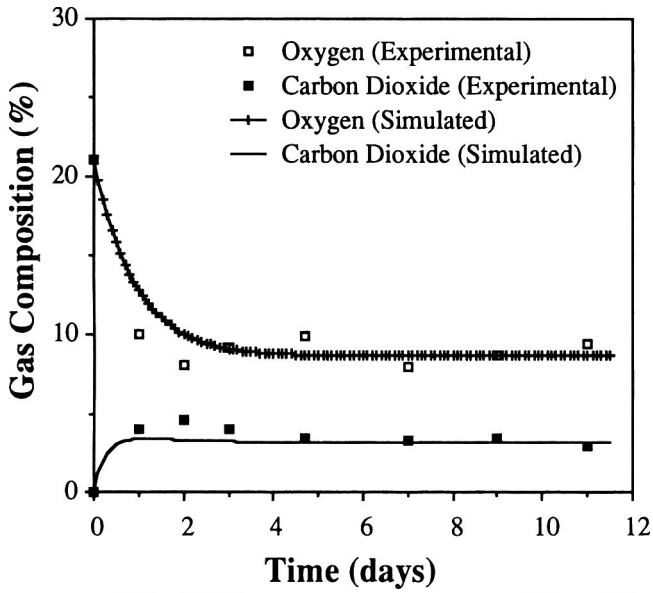

Fig. 3.—Simulated O_2 and CO_2 concentrations inside polyethylene packaged apples compared to experimental data of Jurin and Karel (1963).

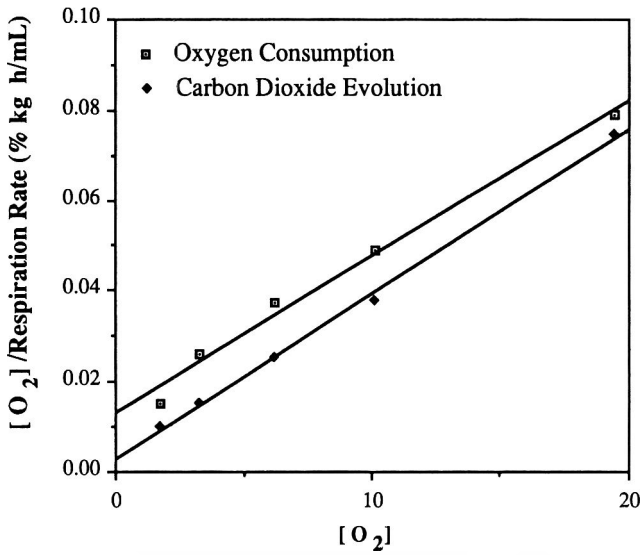

Fig. 4.—Hanes plot for broccoli respiration as a function of O_2 concentration. Solid lines were generated using Eq. (4).

for this purpose, since they included the dependence of respiration on both O_2 and CO_2, and an evaluation of gas composition inside a permeable package over time. V_m was the slope and K_m the intercept of the plot of Eq. (4), Fig. 1, and the parameter K_i was the slope of the plot of Eq. (5), Fig. 2. The estimated values of the parameters from regression analysis were $V_m = 10.80$ mL O_2/kg·h, $K_m = 3.55\%$ O_2, $K_i = 27.98\%$ CO_2. Using these parameters and the package conditions reported by Jurin and Karel (1963), the respiration model was used to simulate the gas environment in a polyethylene bag containing apples. The simulation was done by solving the ordinary differential Eq. (6) and (7) derived previously by Hayakawa et al. (1975).

$$\frac{d[O_2]}{dt} = 100\left(\frac{S\,P_{O_2}\,(0.21-[O_2]/100)}{V\,L} - \frac{W\,r_{O_2}}{V}\right) \quad (6)$$

$$\frac{d[CO_2]}{dt} = 100\left(\frac{S\,P_{CO_2}\,(0.0-[CO_2])/100)}{V\,L} + \frac{W\,r_{CO_2}}{V}\right) \quad (7)$$

These equations were numerically integrated using the Adams-Moulton method in the IMSL subroutine IVPAG (IMSL, Inc., Houston, TX).

Since the free volume inside the package was not reported by Jurin and Karel (1963), it was calculated from the initial slopes of the O_2 and CO_2 change curves using Eq. (6) and (7). The average free volume of the package was calculated to be 405 mL. Figure 3 shows good agreement between the predicted atmosphere concentrations and the experimental data. The equilibrium O_2 and CO_2 concentrations obtained from the simulations (8.69% and 3.17%, respectively) compared well to the values of 8.70% and 3.15%, respectively, reported by Jurin and Karel (1963). Considering the inevitable variability of the data due to separate packages, as mentioned by the authors, the model predicted the transient microatmosphere inside the package and the equilibrium gas composition very well.

Confirmation using cut broccoli data

The effect of O_2 concentration on cut broccoli respiration was analyzed with the Hanes plot (Fig. 4). Again, a strong linearity of the data was observed, which confirmed the applicability of the respiration model. The average standard deviation of the respiration rate data was 34.4 mL/kg·hr for O_2 consumption and 17.3 mL/kg·hr for CO_2 evolution. The slope of the O_2 consumption curve was essentially equal to that of the CO_2 evolution curve, signifying that the R.Q. was not affected significantly by change in O_2 concentration. This same trend was shown by Jurin and Karel (1963) who obtained a straight line with zero slope when R.Q. was plotted vs O_2 concentration.

The effect of CO_2 concentration on the reciprocal respiration rate was shown in Fig. 5, at two constant O_2 concentrations. The inverse of the respiration rate was linear with CO_2 concentration, and the slopes at the two O_2 concentrations were nearly equal. Carbon dioxide evolution rate was greatly affected, but O_2 consumption rate was affected very little by

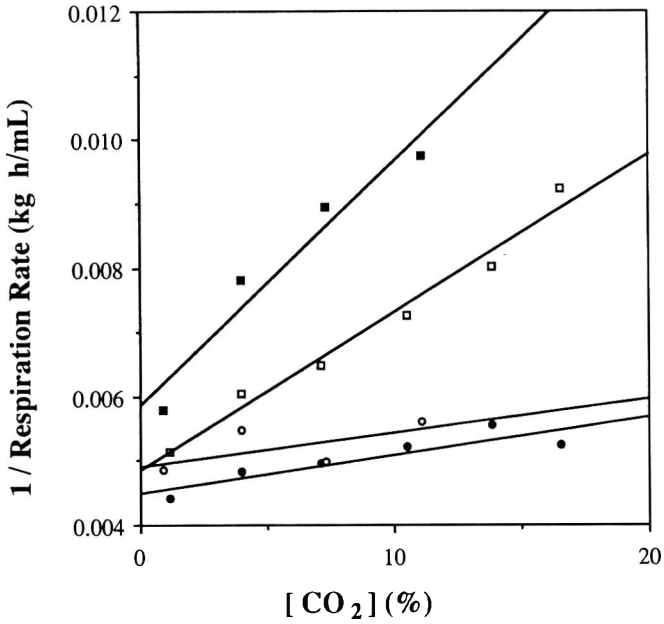

Fig. 5.—Experimental respiration data for broccoli as a function of CO_2 concentration based on a fit by Eq. (5). Oxygen consumption: ●, 21% O_2; ○, [12.5% O_2. Carbon dioxide evolution: □, 21% O_2; ■, 12.5% O_2.]

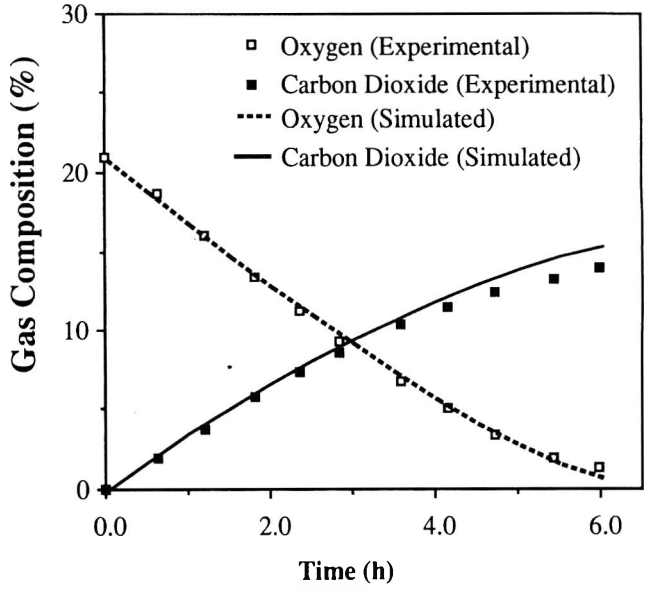

Fig. 6.—Experimental and simulated gas composition inside a model package (2 mil low density polyethylene, 137.3g sample weight, 659 mL free volume, O_2 permeability 198.5 mL·mil/m²·hr·atm, CO_2 permeabiiity 696.0 mL·mil/m²·hr·atm, surface area 0.072 m²) containing broccoli.

Table 3—Respiration rates of cut broccoli at various O_2 and CO_2 concentrations in an open system.

Gas composition		Respiration rates	
$[CO_2]$ (%)	$[O^2]$ (%)	CO_2 evolution rate (mL/kg·hr)[a]	O_2 consumption rate (mL/kg·hr)[b]
0.9	3.4	141.1	140.6
4.6	3.4	121.4	148.2
7.7	3.5	132.7	155.3
10.3	2.6	128.7	141.0
1.1	8.9	182.4	214.3
4.4	9.6	151.0	181.7
7.9	9.6	137.0	173.6
12.1	9.4	131.2	151.4
1.0	16.0	199.1	191.0
4.2	16.5	178.5	232.4
7.3	16.2	158.0	222.8
11.2	16.5	157.6	205.7

[a] Average standard deviation = 16.0 mL/kg·hr
[b] Average standard deviation = 8.2 mL/kg·hr

CO_2 concentration. The average standard deviation of the respiration rate data was 32.2 mL/kg·hr for O_2 consumption and 22.2 mL/kg·hr for CO_2 evolution. The 14.5% CO_2:12.5% O_2 and 17.8% CO_2:12.5% O_2 data points were omitted from the plot because the CO_2 evolution rate was too low to be measured accurately. Although the O_2 level was assumed constant in the experiments, slight changes in the O_2 level might have occurred due to O_2 consumption by the commodity and contributed to variability.

Eq. (5) was fitted to the third set of experiments, which consisted of measuring respiration rates as a function of 12 different gas compositions. The data were analyzed using multiple linear regression (JMP, SAS Institute, Inc. Cary, NC), and the fit was found to be significant at $P<0.001$ (Table 3). The values of the model parameters V_m, K_m, and K_i were estimated: $V_m = 219.4$ mL/kg·hr, $K_m = 1.4\%$ O_2, and $K_i = 114.7\%$ CO_2 for O_2 consumption rate; and $V_m = 191.1$ mL/kg·hr, $K_m = 1.0\%$ O_2, and $K_i = 42.3\%$ CO_2 for CO_2 evolution rate. With these model parameter values, respiration rate as a function of O_2 and CO_2 inside the permeable package were simulated by solving Eq. (6) and (7) numerically. The permeable package, containing 137.3g cut broccoli, was constructed of a 2 mil low density polyethylene package with O_2 permeability of 198.5 mL·mil/m²·h·atm and CO_2 permeability of 696.0 mL·mil/m²·hr·atm, free volume of 659 mL, and surface area of 0.072 m². In general, the predicted gas composition inside the package agreed very well with experimental data, especially for transient gas compositions (Fig. 6). Unfortunately, because of the high respiration rate of cut broccoli and the relatively low O_2 permeability of the film, an equilibrium between permeation and respiration could not be reached prior to the atmosphere inside the package becoming anaerobic. The respiration model is only valid for aerobic respiration, and the gas compositions were not well predicted when the O_2 concentration was very low (Fig. 6).

NOMENCLATURE

$[O_2]$	Oxygen concentration (%)
$[CO_2]$	Carbon dioxide concentration (%)
K_m	Michaelis-Menten constant (% O_2)
K_i	Inhibition constant (% CO_2)
P_{O_2}	Permeability of film to oxygen (mL·mil/m²·hr·atm)
P_{CO_2}	Permeability of film to carbon dioxide (mL·mil/m²·hr·atm)
L	Thickness of polymeric film (mil)
r	Respiration rate (mL/kg·hr or mg/kg·hr)
r_{O_2}	Respiration rate in oxygen consumption (mL/kg·hr or mg/kg·hr)
r_{CO_2}	Respiration rate in carbon dioxide evolution (mL/kg·hr or mg/kg·hr)
V	Package free volume (mL)
V_m	Maximum respiration rate (mL/kg·hr or mg/kg·hr)
S	Surface area of the package (m²)
t	Time (hr)
W	Weight of produce in the package (kg)

CONCLUSIONS

A SIMPLE, semi-empirical respiration model, based on enzyme kinetics principles, adequately described the respiration of fresh produce in terms of O_2 consumption rate and CO_2 evolution rate as a function of O_2 and CO_2 concentrations. The model was confirmed with published data for a wide variety of commodities and with experimental data. The model could

be used in designing MAP systems for fresh fruits and vegetables, provided that the CO_2 concentration is below the tolerance limit of the commodity and sufficient O_2 is available to support aerobic respiration. The model is particularly useful for predicting the transient respiration compositions where temperature fluctuation is encountered, during passive modification of a MAP package, and in similar situations.

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Microbial Changes in Shredded Iceberg Lettuce Stored under Controlled Atmospheres

M.I. BARRIGA, G. TRACHY, C. WILLEMOT, and R.E. SIMARD

ABSTRACT

Our purpose was to determine initial microbial populations in shredded Iceberg lettuce (*Lactuca sativa* L.) cv 'Great Lakes', and to study their changes under different controlled atmospheres. Lettuce was shredded, washed, disinfected, water rinsed and centrifuged before being stored for 12 days under air, 3% O_2, 3% O_2 + 5% CO_2 or 3% O_2 + 10% CO_2. Microbiological analysis and assessment of visual quality were carried out every 3 days. No human pathogens were found. Controlled atmospheres had little or no effect on the microbiological populations studied. A 3% O_2 + 10% CO_2 atmosphere maintained acceptable visual quality of lettuce, without appreciably affecting microbial development.

Key Words: microbes, Iceberg lettuce, minimal processing, controlled atmosphere

INTRODUCTION

MINIMALLY PROCESSED vegetables (MPV) are becoming increasingly popular in Europe and in the U.S.A. They are washed, peeled, sliced or shredded, wrapped raw vegetables stored below 10°C and sold within 8 to 10 days (Nguyen-The and Prunier, 1989). The popularity of such products comes from decreasing the need for washing, trimming or cutting in restaurants and institutions. Transport costs are also reduced by removing waste material, (as high as 40–50%), before shipping (Bolin et al., 1977). The disadvantage of the pre-cut product is that its storage life may be greatly reduced as compared to the intact vegetable. Tissue disruption caused by cutting results in elevated respiration and transpiration, which leads to rapid deterioration. In addition, cutting increases the area of injured tissue available for microbial degradation (Priepke et al., 1976). The most common methods used to improve the storage stability of MPV are low temperature, chemical treatments, modification of pH, modified or controlled atmospheres (MA / CA), or a combination of two or more of these (Shewfelt, 1986; Brackett, 1987; Huxsoll and Bolin, 1989; King and Bolin, 1989).

Chemical treatments have been generally of little value in extending storage life of shredded lettuce (Priepke et al., 1976; Bolin et al., 1977). However, beneficial effects from MA have been reported by Priepke et al. (1976) and Ballantyne et al. (1988) among others. The reported prolongation of shelf life was due mainly to the effect of MA on physiological responses of the plant tissue (Brocklehurst et al., 1987). Although MA can change the general microbial profile of foods (Brackett, 1987), these effects have been less studied. The change reported most often is the proliferation of gram-positive bacteria, and more specifically, lactic acid bacteria (LAB) (Sinell, 1980). The effect of MA on microbial development has been studied in endive (Picoche and Denis, 1988), mixed salads (Chambroy and Nguyen-The, 1988) and mayonnaise-based vegetable salads (Buick and Damoglou, 1989). The latter study showed beneficial effects of 20% CO_2 on spoilage of mixed salads. Chambroy and Nguyen-The (1988) observed little effect of 20% CO_2 on the proliferation of microorganisms but a significant reduction of spoilage. Beuchat and Brackett (1990) studied the effect of low O_2 on microorganisms in shredded lettuce.

The study of the combined effect of high CO_2 and low O_2 on the development of specific microbial groups in cut Iceberg lettuce has been little studied and was the objective of our study. The effects of 3 controlled atmospheres on the visual quality, shelf life and microbial contamination of cut Iceberg lettuce were evaluated. Both food spoilage indicator microorganisms and human pathogens were monitored.

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MATERIALS & METHODS

Lettuce

Iceberg head lettuce cv 'Great Lakes' was obtained locally and used immediately after harvest. Shredded lettuce was prepared by removing outer and damaged leaves and core, and slicing the remaining leaves into 1 cm strips with a knife.

Disinfection and storage conditions

The shredded lettuce was dipped for 1 min in water and disinfected for 5 min in a 100 ppm sodium hypochlorite solution at 15°C. Lettuce was rinsed with water for 2 min and centrifuged for 1 min in a kitchen centrifuge. Residual chlorine was measured by the orthotolidine test. Four plastic containers (40 cm × 60 cm) were ventilated with a humidified stream of air or the desired gas mixture (3% O_2, 3% O_2 + 5% CO_2 or 3% O_2 + 10% CO_2) at 4°C for 12 days. Each container contained 8 trays with 100 g of shredded lettuce. Samples were removed and evaluated every 3 days.

Gas and microbiological analysis

Gas flow was maintained at levels preventing CO_2 accumulation. The atmosphere within containers was tested daily by GC (Gas Partitioner, Fisher-Hamilton). The procedures followed were those outlined by Speck (1984). Culture media, methods and incubation conditions employed are listed in Table 1. For *Salmonella* detection, a 25g sample was added to 225 ml of 0.1% peptone broth and incubated overnight at 37°C. One mL peptonated broth was then transferred into two tubes, one containing 9 mL of tetrathionate broth, and the other containing 9 mL of selenite broth. After overnight incubation

Table 1—Conditions for Microbiological Analysis

Test	Method	Culture media	Incubation Temp (°C)	Incubation Time (days)
Aerobic plate count	pour plate	PCA	35	2
Psychrotrophic microorganisms	pour plate	PCA	4	10
Pectinolytic microorganisms[a]	surface plate	CVP	22	3
Lactic acid bacteria	pour plate	MRS	37 anaerobic conditions	2
Yeasts and molds	surface plate	PDA	22	5
Total coliforms	membrane filtration	MFC	35	1
Fecal coliforms	membrane filtration	TSAM	44	1

[a] Pectinolytic microorganisms were isolated and identificated by the API (NFT) system. A test of pathogenicity was done by inoculating 4 cm lettuce pieces with a 10^8 cells/mL bacterial suspension (Nguyen-The and Prunier, 1989).

at 37°C and 43°C respectively, a loopful of the tube containing tetrathionate broth was streaked onto *Salmonella - Shigella* agar and a loopful from the tube containing selenite broth onto bismuth sulfite agar. Both were incubated at 37°C for 48 hr. At least two colonies suspected as being *Salmonella* or *Shigella* were picked from each plate and streaked onto triple sugar iron agar and lysine iron agar slants. The identity of the colonies was also confirmed with API 20E test strips. A 25g sample of lettuce was added to 225 mL alkaline phosphate buffer (pH 7.6) for testing for *Yersinia enterocolitica.* The buffer was incubated for 21 days at 4°C. After 14 and 21 days 1 mL was streaked onto *Yersinia* selective agar and incubated at 32°C for 24 hr. The identity of potential *Yersinia* was confirmed by triple sugar iron and urea tests and with API 20E test strips.

Visual quality evaluation

Each sample was rated visually by two judges according to the scoring system developed by Kader et al. (1973) for head lettuce, with 9 excellent, 5 the lower limit of sales appeal, and 1 extremely poor. This scoring system included assessment of browning, mechanical damage, wilting and general appearance.

Experimental design

Data were analyzed as a split-plot design with 4 treatments (CA), 4 evaluation dates, 2 replications and 2 blocks corresponding to 2 identical experiments, carried out at 4 days interval, using the analysis of variance (ANOVA) procedures. Duncan's test was done when ANOVA indicated significant differences.

RESULTS & DISCUSSION

Mesophilic aerobic microorganisms

Populations of mesophilic aerobic microorganisms increased from an initial level of 10^4 to 10^7 CFU/g. Increases occurred with storage in all samples regardless of treatment. Priepke et al. (1976), and Beuchat and Brackett (1990) reported that MA (2.25% O_2 + 10.5% CO_2 and 3% O_2 respectively) did not significantly affect populations of mesophilic aerobic microorganisms on shredded lettuce. For MPV, French legislation (there is no current Canadian legislation) allows a maximum of 5×10^4 CFU/g at production stage and 5×10^7 CFU/g at consumption stage (Anonymous, 1988). The microbiological quality could thus be considered satisfactory during the storage period.

Psychrotrophic microorganisms

The levels and evolution of this population were similar to those of mesophilic aerobic microorganisms. The population increased from an initial level of 10^4 to 10^7 CFU/g (Fig. 1). In general, the numbers of psychrotrophic microorganisms found were acceptable. The growth of psychrotrophic microorganisms was not reduced by 3% O_2 with or without 5% CO_2. Beuchat and Brackett (1990) obtained similar results in lettuce stored under 3% O_2; however, in our study, lettuce stored under 3% O_2 and 10% CO_2 contained slightly lower numbers of organisms, and maintained acceptable commercial quality during the storage period. The effect of 3% O_2 and 10% CO_2 was significant ($P \leq 0.05$) on day 9, but was not notable on day 12. A higher CO_2 content might enhance this effect (Buick and Damoglou, 1989).

Among psychrotrophs able to develop in vegetable products, only a few can influence quality during storage (Labadie and Dousset, 1988). The organisms usually responsible for vegetable spoilage are gram-negative bacteria, particularly *Erwinia* and *Pseudomonas* (Lund, 1983; Kraft and Rey, 1979). They are pectinolytic and cause breakdown of the tissue. Consequently, their development is an important factor influencing spoilage. In our study counting these organisms was not possible because of the spreading growth of their colonies. Nevertheless, some pectinolytic strains such as *Pseudomonas luteola, Pseudomonas fluorescens* and *Vibrio fluvialis* were isolated.

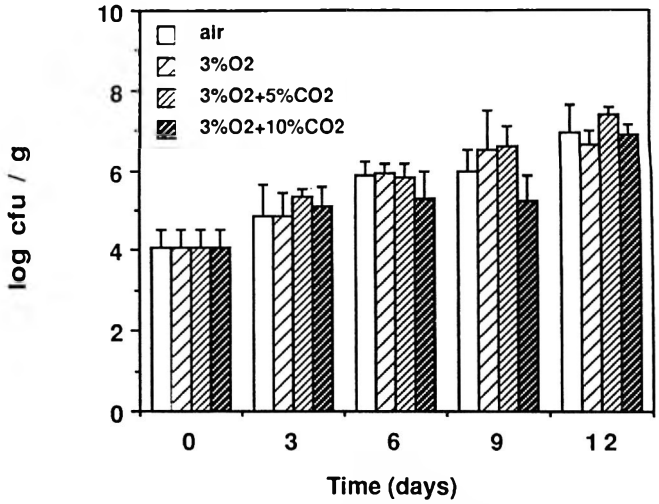

Fig. 1—Evolution of psychrotrophic microorganisms in shredded lettuce stored under different CA for 12 days. Means of 4 determinations ± SD.

The pathogenicity of these microorganisms to the plant was demonstrated by the test described by Nguyen-The and Prunier (1989).

Lactic acid bacteria

The evolution of LAB was somewhat erratic throughout the storage period regardless of treatment. The extremely low level of this population varied between 10^0 and 10^2 CFU/g. This could possibly be explained by competition with other populations, such as pectinolytic microorganisms, which have a higher rate of growth at low temperatures and are better adapted to develop on lettuce because of their pectinolytic enzymes. Other researchers (Chambroy and Nguyen-The, 1988; Picoche and Denis, 1988) have reported that LAB develop well on mixed salads and endive under atmospheres containing 20% CO_2. In both cases, the initial population, which is influenced by harvest season, geographic area and cultivar among other factors, was higher than initial populations we found. LAB are not necessarily destructive to plant tissue (Brocklehurst et al., 1987).

Yeasts and molds

Molds were present occasionally and yeasts were always present but at low levels (10^1 to 10^2 CFU/g). After an increase from 10^3 to 10^5 cfu/g, the yeast population returned to initial levels. Yeasts grow well at neutrality and under alkaline conditions but they do not compete well with bacteria at these pH values (Brackett, 1987). CA did not affect yeast proliferation. Beuchat and Brackett (1990) obtained similar results in lettuce stored under 3% O_2 and Buick and Damoglou (1989) in mayonnaise based vegetable salads stored under 20% CO_2.

Total coliforms

Coliforms, considered as an indication of hygiene at the production stage and the maintenance of the cold chain, were present only in low numbers. Their population increased throughout storage to 10^3 CFU/g. These levels of coliforms are acceptable. CA did not influence their development.

Salmonella, Yersinia and fecal coliforms

No bacteria of public health significance were detected in this study but their presence, even accidental, must not be overlooked. The risk of contamination of vegetables by bac-

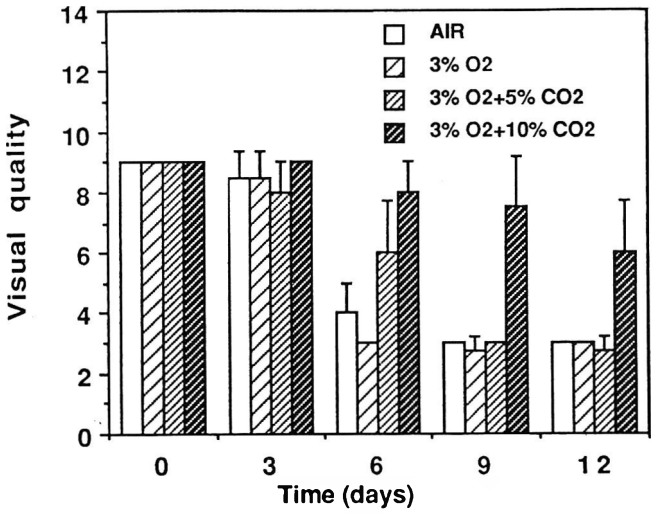

Fig. 2—Visual quality of shredded lettuce stored under different CA for 12 days. Means of 4 determinations.

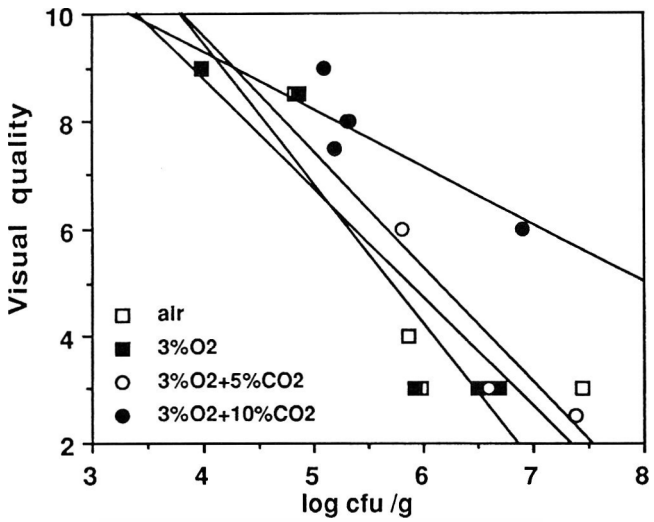

Fig. 3—Relation between visual quality and number of psychrotrophic microorganisms in shredded lettuce stored under different CA for 12 days.

teria of public health significance from soil and irrigation water has been a cause of concern. Several studies have shown that these organisms can be isolated from whole and prepared vegetables. *Staphylococcus aureus, Salmonella* spp. and *Shigella* spp. have been isolated from raw vegetables and salads (Saddik et al., 1985). *Yersinia* spp. and *E. coli* have been isolated from vegetable salad mixes (Brocklehurst et al., 1987). *Listeria monocytogenes* has been detected in MPV (Laine and Michaud, 1988). Pathogens are unlikely to grow on vegetables because normal spoilage flora will usually have a competitive advantage; however, vegetables are not totally without risk (Brackett, 1987).

Visual quality

Visual quality decreased throughout storage, and changes were similar in lettuce stored under air, 3% O_2, and 3% O_2 + 5% CO_2 (Fig. 2). Nevertheless, visual quality was significantly preserved by 3% O_2 + 10% CO_2 ($P \leq 0.05$) which prevented browning. CO_2 is known to prevent browning of damaged plant tissues by blocking production of phenolic compounds, as well as by inhibiting polyphenol oxidase activity (Siriphanich and Kader, 1984). Possibly higher CO_2 levels could have a greater effect on shelf life.

In general, lower psychrotrophic counts correlated with better visual quality (Fig. 3). Browning of injured vegetables is due to a large extent to oxidized phenols acting as an infection barrier (Skovgaard, 1984) and changes in tissue appearance caused by pectinolytic breakdown can be an indication of microbial growth (Wall and Elliot, 1986). However, with 3% O_2 + 10% CO_2, visual quality was maintained despite high microbial counts (10^6–10^7 CFU/g). Both physiological and microbial action can initiate biochemical changes that lead to quality loss of MPV (King and Bolin, 1989). It seems that high CO_2 controlled both microbial and plant enzyme activity without appreciably affecting microbial development (Nguyen-The and Carlin, 1988).

CA has been demonstrated to slow *in vitro* proliferation of phytopathogenic bacteria and reduce soft rot incidence *in vivo* (Barriga et al., 1989; Chambroy and Nguyen-The, 1988; Ibe and Grogan, 1983). However, the *in vivo* effect was probably due to delayed senescence and consequently lesser sensitivity of vegetables to microorganisms (El-Goorani and Sommer, 1981). These effects may also have been due to a direct action on microbial enzyme activity (Nguyen-The and Carlin, 1988), rather than to reduced growth. Microbial proliferation *in vivo* was apparently not affected by the CA tested. Higher CO_2 levels might reduce microbial populations (Buick and Damoglou, 1989).

CONCLUSIONS

VISUAL QUALITY and shelf life of shredded lettuce can be significantly extended by CA (3% O_2 + 10% CO_2), probably by limiting plant and microbial enzyme activity, without appreciably reducing microbial counts. Microbial development in shredded lettuce must be controlled by maintaining low temperatures and other means.

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Ethylene Absorbent to Maintain Quality of Lightly Processed Fruits and Vegetables

KAZUHIRO ABE and ALLEY E. WATADA

ABSTRACT

Ethylene had an undesirable effect on the quality of kiwifruits, bananas, broccoli, and spinach leaves that were prepared and stored as lightly processed products. A 2 or 20 ppm ethylene treatment hastened the softening of the pulp of kiwifruits and bananas held at 20°C. Use of charcoal with palladium chloride, as ethylene absorbent, prevented the accumulation of the ethylene and was effective in reducing the rate of softening in kiwifruits and bananas and of chlorophyll loss in spinach leaves, but not in broccoli.

Key Words: kiwifruit, bananas, broccoli, spinach, ethylene, lightly-processed

INTRODUCTION

QUALITY of lightly processed fruit and vegetable products during handling and storage is maintained by utilizing or modifying practices recommended for fresh produce. Research studies have shown benefits of vacuum cooling (Friedman, 1951), low temperature (Priepke et al., 1976, Bolin et al., 1977, Sugawara et al., 1987), and modified atmospheres (Wolfe and Robe, 1980) for prepackaged spinach, cole slaw, shredded lettuce or mixed salad. Use of washing or chemical treatments to remove exposed cellular components or specific cutting tools to minimize damage has been helpful for shredded lettuce (Bolin et al., 1977; Krahn, 1977, Ohta and Sugawara, 1987).

These practices do not remove ethylene (C_2H_4), a natural ripening initiator that is produced by fruits and vegetables. C_2H_4 is used commercially to ripen climacteric fruits, such as bananas, tomatoes, honeydew melons, and avocados. Since it induces loss of green color (chlorosis), abscission, and softening, commercial storage rooms should have equipment to remove C_2H_4 when potential problems exist (Watada, 1986).

C_2H_4 production can be induced or stimulated when plant tissues are injured (Hoffman and Yang, 1982; McGlasson, 1969), so C_2H_4 production might be expected when fruits and vegetables are peeled, sliced, and cut for preparation as lightly processed products. As these products are placed in sealed packages, C_2H_4 can accumulate and cause undesirable quality changes. We determined the effects of C_2H_4 as well as of an C_2H_4 absorbent on the physiology and quality of kiwifruits, bananas, broccoli, and spinach leaves that were prepared as lightly processed products and stored in trays. Factors monitored were firmness, chlorophyll content, CO_2 and C_2H_4 concentrations, and soluble free amino acids content.

MATERIALS & METHODS

KIWIFRUITS, obtained from a local distribution center, were peeled and sliced to 1.1 cm thickness. Bananas at the green tip stage, obtained from a local supermarket, were cut into 4 cm length sections. Five slices of kiwifruit and five sections of banana were placed together in a metal tray with a glass cover at 20°C with air, or air + 2 or 20 ppm C_2H_4 flowing through the closed tray at 100mL/min. Each treatment was replicated 3× and samples were removed after 0, 1, 2, or 3 days storage for firmness measurements.

The effects of charcoal with palladium chloride to remove C_2H_4 were determined with kiwifruits, bananas, broccoli, and spinach leaves. Broccoli from the supermarket was chopped into small pieces. Spinach from the supermarket was hand-torn into small pieces. Six kiwifruit slices and four sections of banana or 60 g of broccoli pieces and 40 g of spinach pieces were placed in a metal tray with a glass cover at 20°C with or without 10g of paper packets containing C_2H_4 absorbent. Each treatment was replicated 3× and samples were removed after 0, 1, 2, or 3 days storage for determination of chlorophyll and total free amino acids contents in spinach and broccoli tissues and firmness of kiwifruit and banana tissues. CO_2 and C_2H_4 concentrations in each tray were monitored daily.

Firmness was determined with an Instron Model TM by measuring the force required to press a 4 mm × 16 mm flat probe 3 mm into the pulp or core of 1.1 cm thick sliced kiwifruit, or a 4.4 cm diameter round flat probe 5 mm into 1.1 cm thick slice of banana. A single measurement of core tissue and duplicate measurements of pulp tissue were made on each kiwifruit slice. With banana, measurements were made on two slices from each section. Chlorophyll was extracted from spinach leaves or broccoli florets with 80% cold acetone and absorbance of the extract measured at 645 and 663 nm (Arnon, 1949). Free

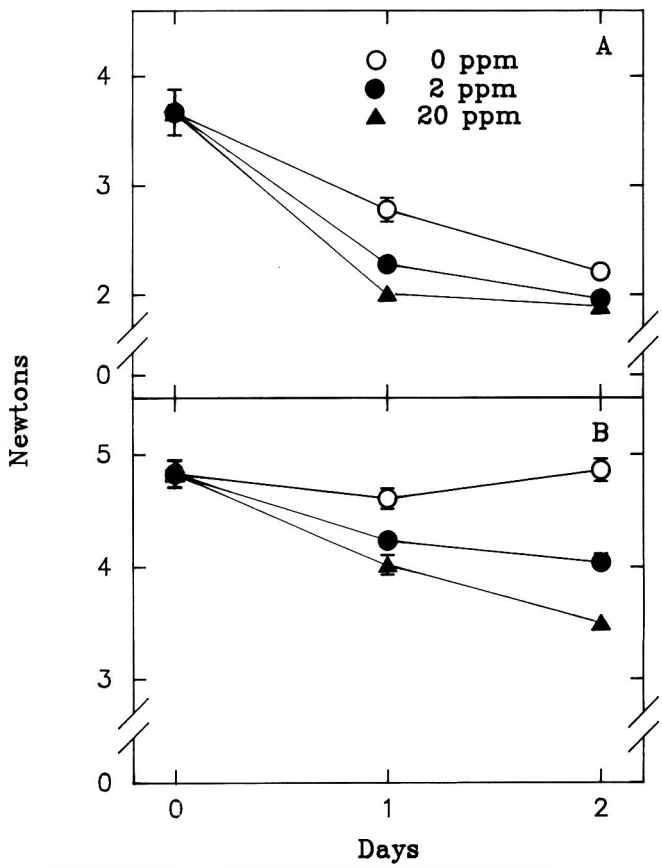

Fig. 1—Firmness of (A) pulp of kiwifruit slices and (B) banana sections held in air with ethylene at 20°C.

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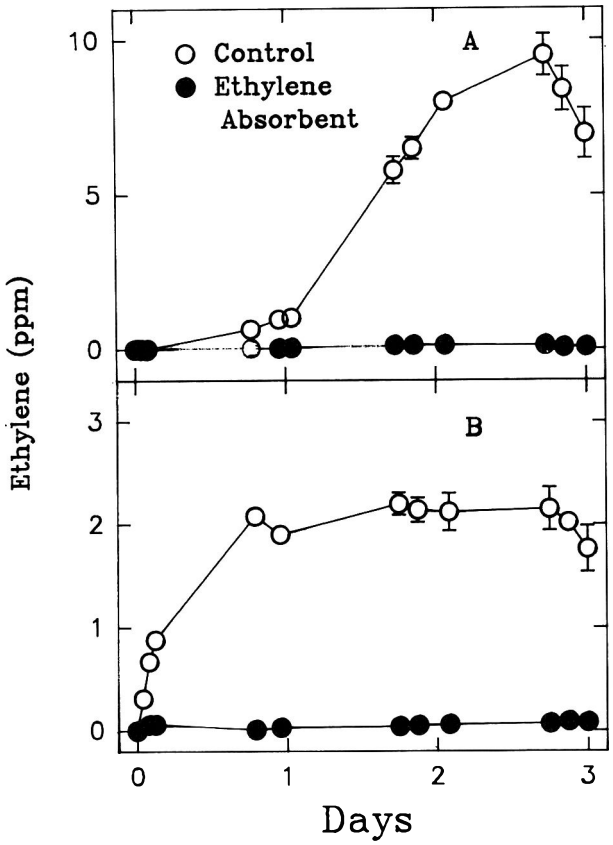

Fig. 2—Ethylene concentration in trays with or without ethylene absorbent (Charcoal with palladium chloride) containing (A) kiwifruit slices and (B) banana sections held at 20°C.

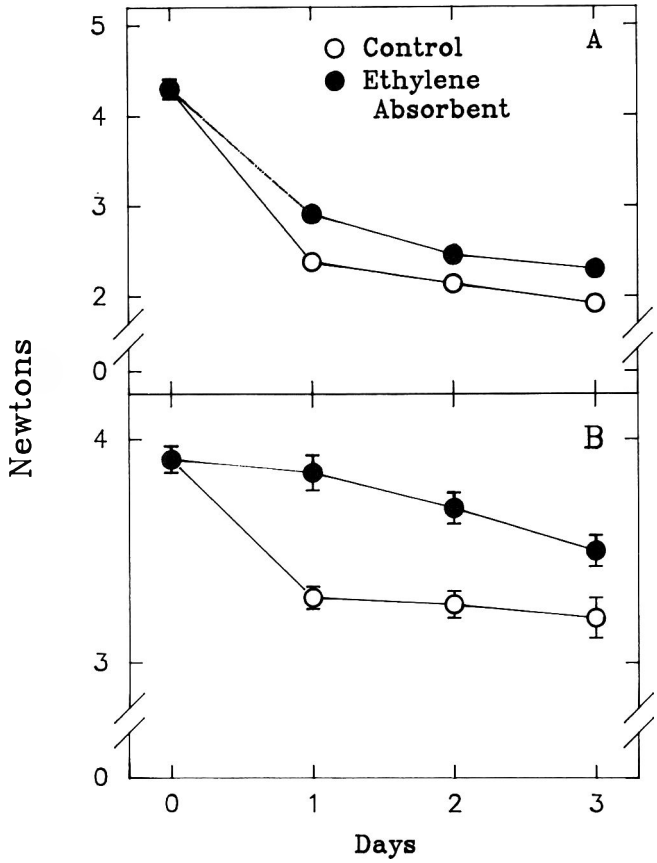

Fig. 3—Firmness of (A) pulp of kiwifruit slices and (B) banana sections stored with or without ethylene absorbent (Charcoal with palladium chloride) and held at 20°C.

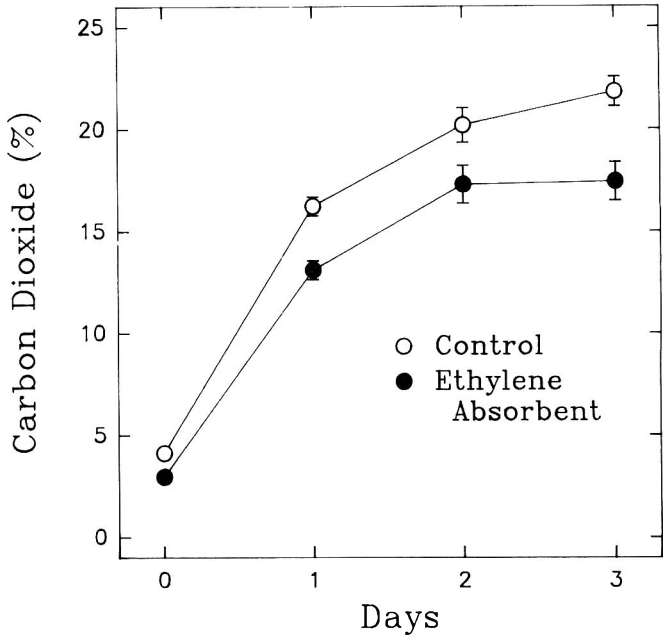

Fig. 4—Carbon dioxide concentration in kiwifruit slices and banana sections trays with and without ethylene absorbent. Trays held at 20°C.

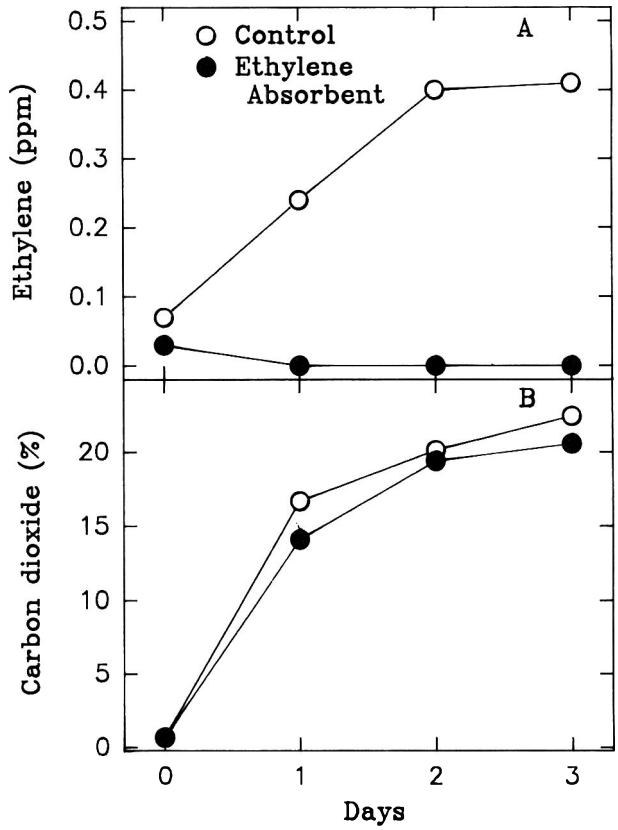

Fig. 5—(A) Ethylene and (B) carbon dioxide contents in broccoli and spinach trays with or without ethylene absorbent stored at 20°C.

amino acids were extracted from spinach leaves or broccoli with hot 80% ethanol, reacted with ninhydrin and hydrindantin in sodium acetate buffer, and absorbance of the reaction product measured at 570 nm (Moore and Stein, 1954). CO_2 and C_2H_4 contents were measured with TCD and FID gas chromatography, respectively.

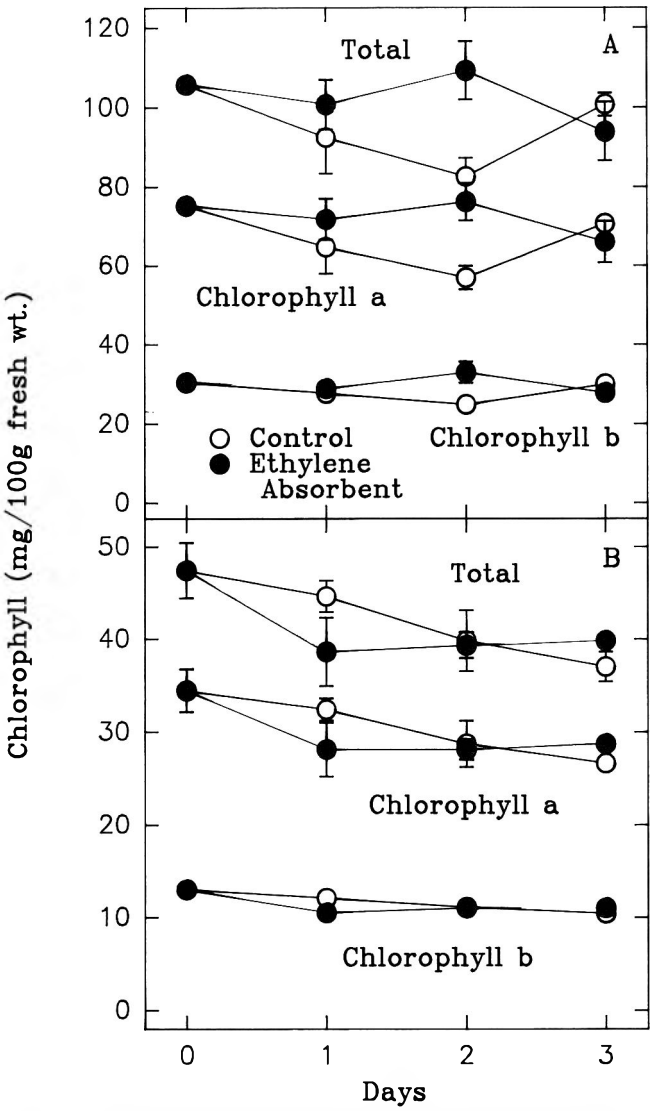

Fig. 6—Chlorophyll content of (A) spinach pieces or (B) broccoli pieces stored with or without ethylene absorbent and held at 20°C.

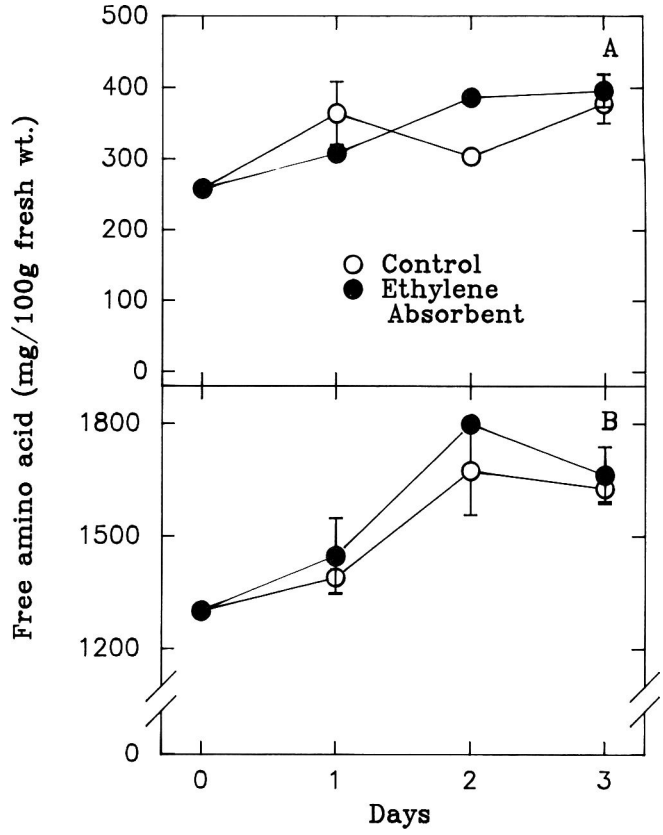

Fig. 7—Free amino acids content of (A) spinach pieces or (B) broccoli pieces stored with or without ethylene absorbent and held at 20°C.

RESULTS & DISCUSSION

THE RATE of softening of sliced kiwifruit pulp and banana sections was enhanced by the 2 or 20 ppm C_2H_4 treatment (Fig. 1). After 1 day at 20°C in air + 2 or 20 ppm C_2H_4, firmness of the kiwifruit pulp decreased by 50%, down to 2 newtons. Firmness of fruit held in air decreased to that level after 2 days storage. Firmness of core tissue was not affected by the C_2H_4 treatment (data not presented). Average firmness of sliced bananas, which initially was about 5 newtons, decreased to 4 and 3.5 newtons with 2 and 20 ppm C_2H_4, respectively, after 2 days storage. Firmness of banana held in air changed minimally during the 3 day period.

Charcoal with palladium chloride was effective in absorbing most of the endogenously produced C_2H_4 during the 3 days storage (Fig. 2). Without the absorbent, C_2H_4 content in the kiwifruit tray had a higher concentration of C_2H_4 than the banana tray. The higher level was due in part to the greater C_2H_4 production by intact kiwifruit than banana fruit and also due to greater cut surface area by the kiwifruit. That is, kiwifruit fruit slices were thin and peeled, whereas the bananas sections were thick and not peeled.

Softening rates of kiwifruits and bananas were affected by the C_2H_4 absorbent (Fig. 3). Firmness of kiwifruit pulp, which initially was about 4.3 newtons, decreased more rapidly without the absorbent than with the absorbent within the first day. Thereafter, rates of softening of both were similar. Firmness of core tissue decreased from about 11 newtons to 5.5 newtons and the rates of decrease of tissue with and without absorbent were similar (data not presented). Bananas without the absorbent softened to a minimum level of about 3.3 newtons by day 1, whereas those with the absorbent softened more slowly to about 3.5 newtons by day 3.

CO_2 accumulated faster in trays without the absorbent than with the absorbent, which probably was due to the C_2H_4 stimulated respiration by the kiwifruits and bananas. (Fig. 4). The 15 to 20% CO_2 level noted in those trays were levels which have been reported to cause injury (Kader, 1986), but no injury was noted, perhaps because of the short holding period. CO_2 has an inhibitory effect on ethylene-induced softening and ripening (Palmer, 1971; Arpaia et al., 1985; Rosen and Kader, 1989).

C_2H_4 in the broccoli and spinach trays had accumulated to about 0.4 ppm, which was effectively absorbed by charcoal with palladium chloride (Fig. 5). Degradation of chlorophyll a was minimized by the reduced level of C_2H_4 in spinach but not in broccoli (Fig 6). In broccoli, degradation of chlorophyll a and b was not affected by the reduced C_2H_4 level, as noted also with chlorophyll b of spinach. Lack of response in broccoli may have been due to its morphological characteristic, that is, broccoli as a floret, does not contain as much active chlorophyll as spinach leaves.

The CO_2 content in broccoli and spinach trays accumulated to about 15% after 1 day and about 20% after 2 days storage (Fig. 5). CO_2, at that high level, probably delayed chlorophyll degradation, as noted with broccoli by Toivonen et al. (1982), and consequently may have masked the maximum effect of the absorbent on chlorophyll degradation and free amino acid changes.

Free amino acids had accumulated in both spinach and broc-

coli and accumulation was not affected by ethylene removal (Fig. 7). Since amino acids accumulate with degradation of proteins during senescence, more accumulation was anticipated in tissues where senescence was hastened by C_2H_4. The time period probably was not sufficient for metabolism to deteriorate with increased senescence.

CONCLUSIONS

C_2H_4 PRODUCTION was stimulated by the physical actions of light processing, and the amount of production was sufficient, even with the vegetables, to have an effect on plant physiology and quality. Removal of C_2H_4 with an absorbent, (charcoal with palladium chloride was very effective) is beneficial for maintaining the quality of lightly processed products.

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C BOTULINUM IN MENHADEN SURIMI. . .From page 1563

means internal temperatures held for specified times, then currently reported surimi time/temperature processing conditions adequately provide a margin of safety with regard to *C. botulinum* type E.

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Nonstarch Polysaccharide Fractions of Raw, Processed and Cooked Carrots

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ABSTRACT

The total and soluble nonstarch polysaccharide (NSP) components of raw, processed (canned and frozen) and home-cooked (boiled) Royal Chantenay carrots have been analyzed. NSP fractions were characterized by separation and summation of the monosaccharides resulting from acid hydrolysis of the parent polysaccharides. Total NSP was primarily composed of glucose, ~37%, and uronic acid, ~35%, containing polysaccharides. Soluble NSP was composed of >50% uronic acids. Processing and simulated home-cooking of raw carrots resulted in an increase in the amount of NSP/unit dry weight. Relative to raw carrots, cooking of canned product resulted in the largest (~twofold) increase in total and soluble NSP/unit dry weight. Relative differences in NSP were not as great when compared on a wet weight basis.

Key Words: polysaccharides, carrots, monosaccharides, hydrolysis, cooking-effects

INTRODUCTION

THE PHYSIOLOGICAL IMPORTANCE of consuming a diet containing fiber is generally accepted. Expert groups from many countries, including the United Kingdom, Canada and Australia, have recommended increased fiber intakes (National Research Council, 1989). The food industry is now producing a range of increased-fiber products in response to public awareness of the potential beneficial effects from dietary fiber. The molecular composition of the fiber in these products is complex, being primarily composed of nonstarch polysaccharides (NSP). In higher plants the NSP include pectins, hemicelluloses and cellulose. Knowledge of the NSP composition of foods is particularly important for ongoing research investigating the putative beneficial effects of dietary fiber. Much of such research is based on surveys of fiber consumption and, therefore, dependent on the accuracy and detail of fiber composition data for different foods.

A recent study of dietary intake data for the U.S. population concluded that the principal source of total dietary fiber in the average U.S. diet was vegetables (Block and Lanza, 1987). This is of particular interest to the food technologist since the influence of commercial processing and home cooking on fiber composition of vegetables has received only limited attention. Studies based on the detergent system of fiber analysis (Robertson and VanSoest, 1981) are in general agreement regarding the effects of cooking and processing on fiber fractions of vegetables. In general, there appeared to be no great loss of neutral detergent fiber (NDF) due to typical home-cooking methods or commercial processing (Zyren et al., 1983). Home-cooking tends to increase the NDF, acid detergent fiber (ADF) and cellulose content of vegetables on a dry weight basis (Mathee and Appledorf, 1978; Herranz et al., 1981; Herranz et al., 1983). Zyren et al. (1983) also found that with exception of squash, there was no readily apparent loss of pectin during home-cooking. A more recent study (Lintas and Cappelloni, 1988) utilizing the AOAC enzymatic-gravimetric method of fiber analysis concluded home-cooking had limited influence on the total dietary fiber (TDF) content of most vegetables. However, there was in general, a shift in fiber composition from insoluble to soluble fraction.

Our objective was to examine processing/cooking effects on the NSP composition of carrots. The study extended the findings of previous investigators by providing a more detailed, quantitative analysis of this major component of dietary fiber.

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MATERIALS & METHODS

Samples and sample preparation

All samples were obtained at a local commercial processing plant. The processed and raw samples were from the same lot of Royal Chantenay carrots. Raw samples were collected directly from the receiving area prior to washing. The carrots to be processed were initially cooked outside prior to peeling. Carrots were then separated by size, the larger carrots (CW6) to be canned and the smaller carrots (CW8) to be frozen. The CW6 carrots were sliced to ~5 mm thickness and packed in 303 cans prior to commercial cooking. Whole CW8 carrots were steam blanched, fast frozen and packaged in bulk. A 10 kg pooled sample was collected for each of the 4 treatments: CW6 raw carrots, CW8 raw carrots, canned, and frozen product.

Each of the 4 treatments was divided in half; one half was analyzed without further treatment and the other after simulated home-cooking. Prior to analysis, each of the pooled samples was homogenized in a Waring blender. All cooked samples and the canned product were drained and homogenized. Subsamples were freeze-dried for moisture determination by difference. All further analyses were then done on the freeze-dried samples. The cooking time varied for each product, determined by the perceived firmness of the "ready-to-serve" product. The cooked-CW6 carrots were prepared from the corresponding raw carrots by washing, peeling, slicing to 5mm thickness, boiling for 5 min and subsequent draining for 5 min. The cooked-CW8 carrots were prepared from the corresponding CW8 raw carrots by washing, peeling, boiling for 15 min and subsequent draining for 5 min. The cooked-frozen carrots were prepared by boiling the frozen product for 15 min followed by 5 min draining. The cooked-canned carrots were prepared by draining the canned product, boiling for 2 min and subsequent draining for 5 min. Following cooking, all samples were homogenized and freeze-dried as described above.

Preparation of total NSP

The sample preparation procedure for total NSP was adapted from the method of Englyst and Cummings (1988). To a 150–200 mg freeze-dried sample was added 2 mL dimethylsulfoxide and the resulting suspension immersed in a 100°C bath for 1 hr. Sodium acetate buffer (8 mL, 0.1M, pH 5.2) was then added followed by addition of 39 mg pancreatin (Sigma Chem. Co.) in 0.5 mL reaction buffer and 80 μL of pullulanase solution (pullanase suspension, EC 3.2.1.4, Boehringer Mannheim Biochem., diluted 1:100 with water). The resulting suspension was stored in a 42°C bath for 16 hr with periodic shaking. Solubilized NSP was then precipitated by addition of ethanol to 80% and the residue separated by centrifugation. The residue was washed twice with 85% ethanol, then with acetone, and dried under vacuum.

Preparation of soluble NSP

The extraction procedure is described schematically in Fig. 1. A 300-350 mg sample was dispersed in 10 mL water containing 30 mg pepsin, the pH adjusted to 1.5 with HCl, and the suspension incubated with orbital agitation at 38°C for 1 hr. Ten mL of 0.1M Na-phosphate buffer, pH 6.8, containing 30 mg pancreatin was then added, the pH readjusted to 6.8, a few crystals of thymol added and incubation continued another 16 hr. The incubation was terminated by filtering the

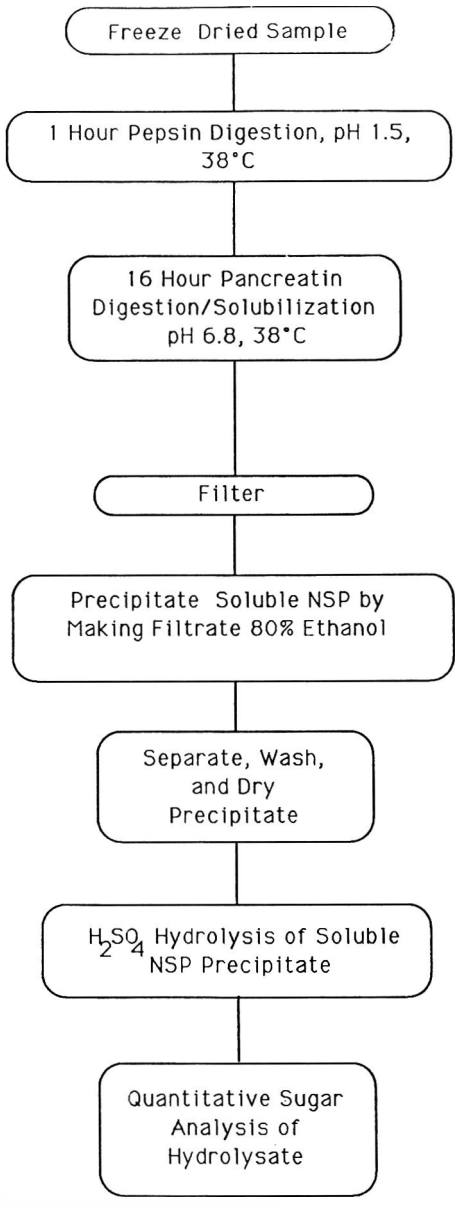

Fig. 1—Schematic of soluble nonstarch polysaccharide (NSP) extraction and analysis.

sample, washing the retentate with 10 mL incubation buffer and combining the filtrates. The pH of the combined filtrate was then adjusted to 5.2, ethanol was added to a final concentration of 80% (v/v) and the precipitate allowed to settle for 1 hr. The precipitated soluble fiber was separated by centrifugation, washed and dried as described.

Analysis of component sugars and moisture

The total and soluble NSP residues were hydrolyzed with sulfuric acid using the primary and secondary hydrolysis scheme of Englyst and Cummings (1988). Neutral sugars in the hydrolysate were derivitized to alditol acetates and separated by gas liquid chromatography (Englyst and Cummings, 1988). Quantitation of constituent sugars was achieved by utilizing relative response factors determined by the internal standard method. Sugar standards were purchased from Pfanstiehl Laboratories, Inc., Waukegan, IL., and myoinositol was used as internal standard. Uronic acids were quantified by the spectrophotometric method of Scott (1979).

Gas liquid chromatography was performed on a Varian 1400 chromatograph equipped with a flame ionization detector, an HP 3380A integrator and a 2m × 2 mm i.d. glass column packed with 3% SP-2340 on 100/120 Supelcoport (Supelco, Bellefonte, PA). Chromatography operating conditions were: column temperature, 225°C; detector, 275°C; carrier gas (nitrogen) flow rate, 15 mL/min. Moisture determinations were done on representative samples drying at 70°C to constant weight (~16 hr).

Statistical analyses

Differences in content of neutral sugars, uronic acids, total NSP and soluble NSP between treatments were determined by one way analysis of variance at the 99% confidence level. Relative changes in the polysaccharide composition of the total NSP and soluble NSP fractions were analyzed by two way analysis of variance. Statistical analyses were done with the SAS statistical analysis program (Anonymous, 1982).

RESULTS

Total nonstarch polysaccharides

The total nonstarch polysaccharide content of the carrot preparations are presented in Table 1. The CW6 and CW8 carrots differed in size but were from the same lot. The larger carrots, CW6, were used to produce the sliced canned carrots. The frozen product consisted of the smaller carrots, CW8, frozen whole. The two raw carrot preparations were not significantly different, each containing about 21g of total NSP/100 g dry matter. The NSP content of these carrots was similar to the value reported previously for unidentified varieties of carrots (Englyst et al., 1988). Processing and simulated home-cooking significantly influenced amount of NSP/unit dry weight. The canning and freezing processes each resulted in a relatively small increase in total NSP per unit dry weight, about 28% and 12%, respectively. The effect of cooking on total NSP fraction was dependent on the carrot preparation. Cooking resulted in a greater increase in the total NSP/unit dry weight of raw sliced carrots (70%) than for raw whole carrots (32%). Simulated home cooking of the processed carrots resulted in the largest increase in total NSP relative to raw product. Cooking the canned product nearly doubled its total NSP content/unit dry weight relative to the original raw CW6 carrots. The total NSP content of the smaller whole carrots, CW8, was increased to the greatest extent by cooking of the frozen product.

The principal sugars that comprised the total NSP were glucose and uronic acids, with lesser amounts of galactose and arabinose (Table 1). This indicated a high proportion of the NSP was composed of pectic polysaccharides and cellulose. These results agreed with those of Stevens and Selvendran (1984), who concluded that the preponderant polymers of carrot cell walls were pectic polysaccharides with associated galactans and arabinans. The statistical data (Table 2) indicate that the increases in total NSP due to processing and cooking were, in general, due to a nonselective increases in all of the constituent polysaccharides. The percent difference (residuals) between the ANOVA predicted values, based on the ratio given in the final column of Table 2, and the measured values, given in Table 1, was generally less than 10%. The exception to this was the value for uronic acids. The large negative residual for the canned, (~17.1%), and the canned-cooked, (~24.9%), uronic acids may reflect a loss of pectic polysaccharides relative to the other NSP. The apparent loss of pectic polysaccharides appears related to the commercial cook/sterilization process that is unique to the canned products. The large negative residuals for the uronic acids and the negative, although somewhat smaller, residuals for rhamnose in canned products suggested some leaching of rhamnogalacturonan polymers during commercial sterilization.

Soluble nonstarch polysaccharides

The isolation scheme used to prepare the soluble NSP fraction, (Fig. 1), simulated digestive processes in the intestinal tract, thereby providing a physiologically relevant estimate of soluble fiber content. The soluble NSP content of the different preparations varied in a manner similar to that of total NSP

Table 1 – Total nonstarch polysaccharides in carrots[a]

Sample	Composition, g/100g dry wt							
	Total	Rham	Ara	Xyl	Man	Gal	Glu	Uac
Raw CW6	20.2[f]	0.44[a]	1.43[d]	0.41[f]	0.46[a]	3.05[d]	7.40[a]	7.07[de]
Cooked CW6	34.5[b]	0.74[ab]	2.36[b]	0.70[bc]	0.79[b]	4.87[b]	12.57[b]	12.46[a]
Raw CW8	21.7[f]	0.46[de]	1.41[d]	0.46[ef]	0.54[de]	2.94[d]	8.32[de]	7.57[cd]
Cooked CW8	28.7[d]	0.62[c]	1.89[c]	0.66[bc]	0.71[bc]	3.79[c]	11.03[c]	10.00[b]
Canned CW6	25.8[e]	0.50[de]	1.96[c]	0.62[cd]	0.64[cd]	4.11[c]	11.20[bc]	6.81[a]
Cooked Canned	40.1[a]	0.77[a]	3.11[a]	1.03[a]	1.04[a]	6.43[a]	18.04[a]	9.68[b]
Frozen CW8	24.4[e]	0.52[d]	1.90[c]	0.55[de]	0.52[ae]	3.88[c]	9.19[d]	7.83[c]
Cooked Frozen	31.9[c]	0.68[b]	2.54[b]	0.73[b]	0.69[bc]	5.20[b]	12.15[bc]	9.91[b]
CV (%)	3.8	5.6	9.0	6.5	10.0	4.6	6.3	4.0
LSD	2.13	0.07	0.37	0.09	0.14	0.40	1.41	0.70

[a] Means in columns not sharing a common superscript letter are significantly different, $p<0.01$.
Rham = rhamnose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glu = glucose; Uac = uronic acid

Table 2 – Comparison of ANOVA derived and measured total nonstarch polysaccharides in carrots[a]

Sample	Composition, g/100g dry wt								
	Total	Rham	Ara	Xyl	Man	Gal	Glu	Uac	Ratio
Raw CW6	19.9	0.42	1.44	0.45	0.47	2.98	7.80	6.28	1.000
Residual	1.5%	5.5%	−0.7%	−9.2%	−2.3%	2.2%	−5.1%	12.4%	
Cooked CW6	33.8	0.70	2.45	0.76	0.80	5.06	13.22	10.66	1.697
Residual	1.9%	5.0%	−3.5%	−7.9%	−1.0%	−3.9%	−4.9%	16.8%	
Raw CW8	21.4	0.45	1.55	0.48	0.50	3.20	8.37	6.75	1.074
Residual	1.3%	3.8%	−8.8%	−3.1%	6.4%	−8.3%	−0.6%	12.2%	
Cooked CW8	28.6	0.60	2.07	0.64	0.67	4.27	11.16	9.00	1.432
Residual	0.4%	3.4%	−8.4%	3.6%	5.4%	−11.3%	−1.2%	11.0%	
Canned CW6	26.1	0.54	1.89	0.58	0.61	3.90	10.19	8.22	1.307
Residual	−0.9%	−8.8%	4.1%	5.8%	4.9%	5.3%	10.0%	−17.1%	
Cooked Canned	40.9	0.85	2.96	0.92	0.96	6.12	15.98	12.89	2.051
Residual	−2.0%	−9.2%	5.2%	12.7%	7.6%	5.1%	12.9%	−24.9%	
Frozen CW6	24.5	0.51	1.77	0.55	0.58	3.67	9.58	7.73	1.229
Residual	−0.6%	1.0%	7.4%	0.2%	−9.0%	5.6%	−4.1%	1.3%	
Cooked Frozen	32.3	0.67	2.34	0.72	0.76	4.84	12.63	10.18	1.620
Residual	−1.3%	1.3%	8.6%	1.0%	−9.7%	7.6%	−3.8%	−2.7%	

[a] Values given are predicted based on two-way ANOVA. Residuals represent the % difference in the actual measured values (Table 1) and those predicted. Rham = rhamnose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glu = glucose; Uac = uronic acid

Table 3 – Soluble nonstarch polysaccharides in carrots[a]

Sample	Composition, g/100g dry wt							
	Total	rham	ara	xyl	man	gal	glu	uac
Raw CW6	5.4[a]	0.10[a]	0.86[da]	tr	tr	1.56[d]	tr	2.84[a]
Cooked CW6	8.8[c]	0.17[c]	1.08[c]	tr	tr	2.22[c]	tr	5.39[c]
Raw CW8	5.2[a]	0.10[a]	0.71[a]	tr	tr	1.52[d]	tr	2.91[a]
Cooked CW8	9.5[c]	0.18[d]	1.00[cd]	tr	tr	2.20[c]	tr	6.11[b]
Canned CW6	9.4[c]	0.20[c]	1.32[b]	tr	tr	2.77[b]	tr	5.15[c]
Cooked Canned	13.6[a]	0.39[a]	2.03[a]	tr	tr	4.17[a]	tr	7.00[a]
Frozen CW8	6.8[d]	0.12[e]	0.84[de]	tr	tr	1.94[cd]	tr	3.93[d]
Cooked Frozen	11.1[b]	0.24[b]	1.32[b]	tr	tr	2.96[b]	tr	6.61[ab]
CV(%)	3.9	6.0	6.9			8.9		4.6
LSD	0.81	0.03	0.19			0.52		0.56

[a] Means in columns not sharing a common superscript letter are significantly different, $p<0.01$.
Rham = rhamnose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glu = glucose; Uac = uronic acid

(Table 3). The cooked-canned, cooked-frozen, and the sliced, cooked carrots (CW6) were highest in soluble NSP/unit dry weight. The cooked-canned preparation had about 2.5-fold more soluble NSP than the corresponding raw starting material. This compared with a twofold increase in the total NSP for the same two preparations.

The principal sugars that comprised the soluble NSP of carrot in all of the preparations studied were uronic acids, galactose, arabinose and rhamnose (Table 3). The sugar composition confirmed the preponderance of pectic polysaccharides in the soluble NSP fraction. The sugar composition could be rationalized by considering that carrot contains significant quantities of rhamnogalacturonan polysaccharides as well as other pectic polysaccharides containing arabinose and galactose (Stevens and Selvendran, 1984). Similar to the measures of total NSP, the sugar composition of the soluble NSP from different preparations indicates a nonselective increase in soluble NSP fraction of the processed carrots relative to raw carrots. This was evident when we compared the residuals resulting from comparison of measured data (Table 3) and the values obtained by ANOVA based on ratios given in Table 4. The data suggested the presence of the same polysaccharides in the soluble fractions of each preparation. However, the actual polysaccharide composition could not be derived from the presented data. Further studies are required to definitively determine the compositional changes occurring in the soluble NSP fraction due to commercial processing and cooking. The high residual for rhamnose in the canned-cooked preparation relative to the canned preparation or the cooked preparation was indicative of the complexity involved in following processing effects on this group of polysaccharides.

Nonstarch polysaccharide content per serving size

The relative amount of fiber in the carrot preparations was different when considered on a wet weight basis (Table 5).

Table 4—Comparison of ANOVA derived and measured soluble nonstarch polysaccharides in carrots[a]

Sample	Composition, g/100g dry wt								
	Total	Rham	Ara	Xyl	Man	Gal	Glu	Uac	Ratio
Raw CW6	5.5	0.11	0.71	tr	tr	1.50	tr	3.11	1.000
Residual	−1.9%	−8.9%	21.3%			3.5%		−8.7%	
Cooked CW6	8.5	0.18	1.11	tr	tr	2.34	tr	4.86	1.560
Residual	3.8%	5.1%	−2.7%			−5.1%		10.9%	
Raw CW8	5.2	0.11	0.68	tr	tr	1.44	tr	2.98	0.957
Residual	0.0%	−5.6%	4.0%			5.8%		−2.4%	
Cooked CW8	8.8	0.18	1.15	tr	tr	2.43	tr	5.03	1.616
Residual	7.3%	−1.7%	−13.4%			−9.5%		21.5%	
Canned CW6	9.7	0.20	1.27	tr	tr	2.67	tr	5.54	1.779
Residual	−2.9%	3.0%	3.9%			3.6%		−7.0%	
Cooked Canned	15.0	0.31	1.95	tr	tr	4.11	tr	8.51	2.735
Residual	−9.1%	27.1%	4.2%			1.4%		−17.8%	
Frozen CW8	6.6	0.14	0.86	tr	tr	1.82	tr	3.78	1.213
Residual	3.0%	−9.6%	−3.4%			6.6%		4.0%	
Cooked Frozen	11.0	0.22	1.44	tr	tr	3.02	tr	6.27	2.013
Residual	1.1%	4.4%	−8.1%			−2.0%		5.4%	

[a] Values given are predicted based on two-way ANOVA. Residuals represent the % difference in the actual measured values (Table 1) and those predicted. Rham = rhamnose; Ara = arabinose; Xyl = xylose; Man = mannose; Gal = galactose; Glu = glucose; Uac = uronic acid

These relative changes were due to differences in moisture content of the carrot preparations. Measured moisture contents with standard deviations of 0.1% were raw CW6, 90.5, cooked CW6, 92.1, raw CW8, 91.0, cooked CW8, 91.4, canned, 92.5, cooked-canned, 94.1, frozen, 89.4, and cooked-frozen carrots, 91.1. The cooked frozen carrots contained the highest amount of total NSP and soluble NSP/serving size. The raw carrot preparations contained the lowest amount of soluble NSP/serving and they, along with the canned carrots, contained the lowest total NSP/serving size. In general, quantitation of NSP on a wet weight basis resulted in reduced differences between preparations. For example, the ratio of total NSP in the cooked-canned carrots and the corresponding raw carrots was 1.98 on a dry weight basis and 1.22 on a wet weight basis. Further, considering the extreme values, there was nearly a 2-fold difference in total NSP on a dry weight basis compared to 1.5-fold on a wet basis. Similarly, the extreme values for soluble NSP differed by 2.6-fold on a dry weight basis and 2.1-fold on a wet weight basis.

The ratio of soluble to total NSP/serving is similar for the different preparations (Table 5). The raw carrots had the lowest ratio, 0.26, while the canned, 0.36, cooked-canned, 0.34, and cooked-frozen, 0.35, had the highest ratios. The data suggested that heat treatments may help solubilize a portion of the total NSP. However, the cooked CW6 carrots demonstrated that the relationship of cooking to soluble NSP is not simple. The ratio of soluble to total NSP remained the same as that of corresponding raw carrots.

Table 5—Nonstarch polysaccharide content per 100g serving (~ 1 cup) of carrots[a]

Sample	Total NSP (g)	Soluble NSP (g)	Soluble NSP / Total NSP
Raw CW6	1.92[a]	0.51[a]	.26
Cooked CW6	2.70[ab]	0.69d	.26
Raw CW8	1.94[a]	0.47[a]	.24
Cooked CW8	2.46[cd]	0.81[b]	.33
Canned CW6	1.93[a]	0.70[d]	.36
Cooked canned	2.35[d]	0.80[bc]	.34
Frozen CW8	2.59[bc]	0.73[cd]	.28
Cooked Frozen	2.84[a]	0.99a	.35
CV(%)	3.6	4.1	
LSD	0.17	0.08	

[a] Means in columns not sharing a common superscript letter are significantly different, $p<0.01$.

DISCUSSION

PROCESSING and cooking of carrots clearly increased their amount of NSP/unit dry weight. It is extremely unlikely that increases in NSP we observed were due to the actual formation of new NSP. The increase in percent NSP was likely due to leaching of the more readily solubilized solids. Aside from leaching, processing of food products can result in an increase in apparent dietary fiber due to formation of products like lignin (Hartley, 1978), which is a component of dietary fiber. However, such products would not influence results of our study which are based on the specific analysis of NSP.

The results of our study were in general agreement with the findings of Matthee and Appledorf (1978) that cooking caused a significant increase in the percent ADF and crude fiber of carrots on a dry weight basis. In the same study it was observed that the NDF fraction increased similarly after more prolonged cooking. Zyren et al. (1983) also reported an increase in the NDF fraction of frozen and canned carrots due to cooking, their results being analyzed on a wet weight basis. In contrast to those studies, Herranz et al. (1981) observed no increase in NDF or ADF fractions of carrots due to cooking on either a wet or dry weight basis. Lintas and Cappelloni (1988) similarly found essentially no effect of cooking on total dietary fiber (Prosky et al., 1985) content of carrots. Our results agree with those of Lintas and Cappelloni (1988) who observed a relative increase in soluble to insoluble fiber ratio of cooked carrots. Englyst et al (1988) reported the NSP content of raw, cooked and canned carrots from supermarkets. Their data indicated an apparent increase in total NSP due to cooking, analyzed on a dry weight basis, but no apparent difference in total NSP analyzed on a wet weight basis. Comparison of our soluble NSP data with those of Englyst et al. (1988) demonstrated the significance of the extraction procedure used to obtain soluble NSP. The soluble NSP values reported in our study for raw carrots were about 60% less than the corresponding values reported by Englyst et al. (1988). Soluble NSP in our study was defined by the extraction procedure of Figure 1, while in the other study it was defined as the NSP solubilized in phosphate buffer at 100°C.

Our results indicated that individuals consuming different types of carrot preparations are likely to consume different quantities of total and soluble fiber/serving. This is of relevance to the large number of studies which correlate fiber intake of populations and incidence of chronic disease. Differences in fiber content of the preparations were dependent on whether the carrots were compared on a wet or dry weight basis. Consequently, to interpret the published values of NSP

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Equilibrium Water Content and the State of Water in Dehydrated White Cabbage

KIRSTI PÄÄKKÖNEN and LIISA PLIT

ABSTRACT

The water sorption isotherms for freeze-dried cabbage were determined at 15°C and 23°C, and the state of the water was studied using differential scanning calorimetry (DSC). The water sorption capacity of the freeze-dried cabbage depended on the temperature. The effects of free sugars were clear in both adsorption and desorption. The thermal behavior of freeze-dried cabbage was highly moisture dependent. In the capillary condensation range (>0.9 a_w) solution of low molecular components of the freeze-dried cabbage affected its freezing behavior.

Key Words: cabbage, water activity, dehydration sorption isotherm

INTRODUCTION

RETAINED WATER ACTIVITY is the main criterion for the stability of dried foods containing sugar. The water sorption isotherm and thermal behavior combined should be used to determine proper drying and storage conditions. Retention of aroma during drying and storage of dried carbohydrate materials has been related to structural changes (Flink and Karel, 1970; Tsourouflis et al., 1976; To and Flink, 1978). The sugars contained in food materials may be either bound to other components or free in different physical states. The crystallization of amorphous sugars has been associated with the rate of browning, stickiness and caking. Mizrahi et al. (1970) studied the sorption capacity and extent of browning of freeze-dried cabbage in storage tests.

The surface temperature during freeze-drying has been found to change the shape of the sorption isotherms of carbohydrate materials (Pääkkönen and Roos, 1990). The objective of our study was to obtain more data on the water-binding capacity of carbohydrate materials by studying the water sorption and thermal behavior of freeze-dried cabbage.

MATERIALS & METHODS

Preparation of samples

Cabbage (*Brassica oleracea* var. capitata "f. alba") were purchased from a local dealer and cut into 2 cm slices before being frozen in a flow-freezer at −45°C. Shelf temperature in the freeze-dryer (Edwards EF 10/10 U.K.) was 35°C, and the pressure was <20 Pa. The freeze-dried cabbage was powdered and packed in aluminum laminate bags under vacuum. For the experiments, the cabbage powder was removed from the bags and dried for 2 wk in a desiccator over P_2O_5.

Determination of sorption isotherms

To ensure that the sample was as dry as possible, 50 mg sample were kept in a dish in a vacuum desiccator over P_2O_5 until a constant weight was reached: after creating a vacuum the lid of the dish was opened and the desiccator was left to evacuate. The sample dish was closed before releasing the atmospheric pressure into the desiccator (Pääkkönen and Kurkela, 1986). The gravimetric water sorption method permitted observation of sample weight until equilibrium was reached (Pääkkönen, 1987). The adsorption and desorption isotherms were determined at 15°C and 23°C by interval sorption, exposing the same sample stepwise to atmospheres of increasing relative humidities, achieved with saturated salt solutions (LiBr, LiCl, $KC_2H_3O_2$, $MgCl_2$, K_2CO_3, $Mg(NO_3)_2$, NaBr, NaCl, Li_2SO_4) (Rockland, 1960). Equilibrium was reached in 1-3 hr. In desorption the same sample was allowed to equilibrate at different humidities, but in reverse order. A slow, thermostatically controlled air stream (15 mL/min) was bubbled through the saturated salt solutions. The temperature of the equilibrium flask was regulated by circulating thermostatically controlled liquid around the flask.

The humidity was continuously controlled by a Humicap HMI 14, capacitative, thin film, relative humidity tester with an HMP 14 sensor (Vaisala Ltd., Finland). The isotherms were determined in at least duplicates (Pääkkönen, 1987).

Differential scanning calorimetry

A differential scanning calorimetry (DSC) system (Mettler TA 3000) and a DSC 30 measuring cell was used. For measurements, weighed samples of powdered, freeze-dried cabbage were humidified in pans for 14 days over saturated salt solutions (LiCl, $KC_2H_3O_2$, K_2CO_3, $Mg(NO_3)_2$, NaCl, $(NH_4)_2SO_4$, KCl). After equilibration the pans were hermetically sealed and reweighed. The samples were first cooled to −170°C and kept at that temperature for 10 min. Samples were then heated to 80°C, at 10°C/min, and thereafter cooled to −170°C. This cycle was repeated twice. The amount of freezable water was calculated by comparing the area of the melting peak of the first test to that of pure water. The amount of unfrozen water was calculated as the difference between total water content and the amount of freezable water calculated from the thermograms. The dry weight of the cabbage was estimated by heating separate samples, placed in Al-pans with perforated lids, in the calorimeter at 105°C until constant weight was achieved. A water content of 8.9% was found.

RESULTS & DISCUSSION

FRESH WHITE CABBAGE had a high moisture content, 92%, and a low fiber content, 2.0%. Available carbohydrates content was 3.4%, all of which was free sugars: 1.4% fructose, 1.9% glucose and 0.1% sucrose (Varo et al., 1984).

The sorption isotherm of a polymer is known to have a regular sigmoidal shape. However, the sorption isotherm of cabbage had an irregular shape, caused by its low fiber content. Cabbage is rich in low molecular weight sugars, in which physical changes are known to occur during freeze-drying i.e. their amorphous state is a consequence of the conditions prevailing during freeze-drying. Further, the amorphous structure of low molecular weight substances is not a permanent state but will change due to water uptake in adsorption, and will partially reorganize in desorption. As a consequence, the shape of the desorption curve was different from that of the adsorption curve.

The adsorption and desorption isotherms of the freeze-dried cabbage determined at 15°C and 23°C are shown in Fig. 1. The irregular shape of the sorption isotherms indicated that in freeze-dried cabbage the solids were in an amorphous metastable state which was very sensitive to changes in temperature and moisture content. The paths of the adsorption and desorption isotherms were different at 15°C and 23°C, with the exception that at 23°C the water capacities in adsorption and in desorption were equal in the range of 0.2 to 0.3 a_w. Within this a_w range, the adsorption capacity was higher at 23°C than

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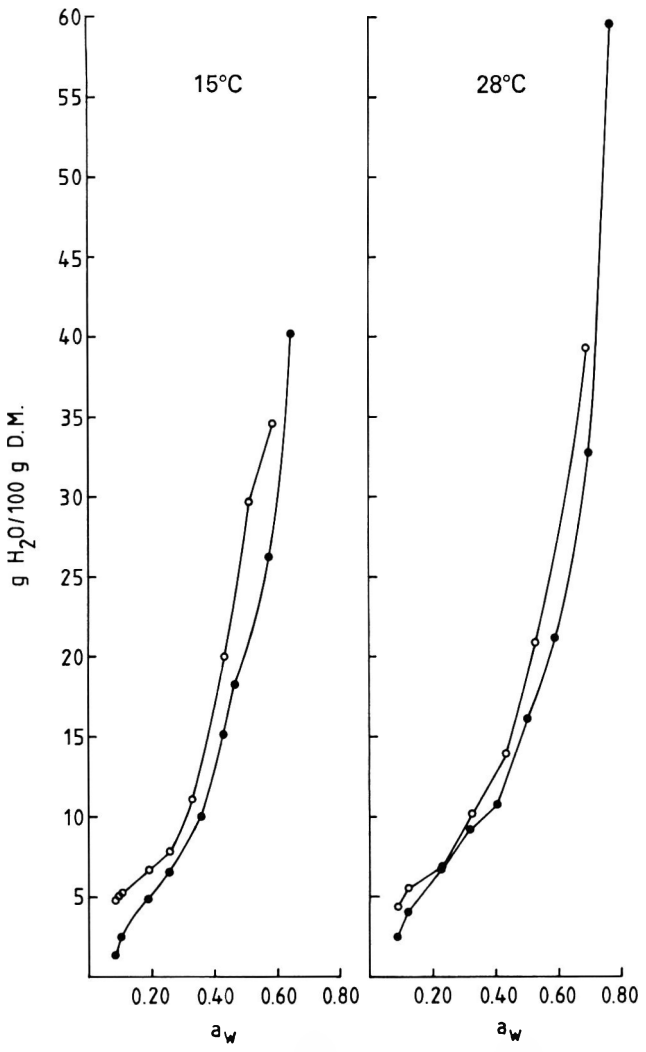

Fig. 1.—Adsorption (●) and desorption (○) isotherms of freeze-dried cabbage at 15°C and 23°C.

at 15°C, contrary to the expected effect of temperature on sorption isotherms; i.e. as the temperature increased, the quantity of water at a given relative humidity decreased. This may have been caused by differences in water sorption of free sugars (Mazza, 1984; Makower and Dye, 1956). In desorption the changes in water binding capacity of the cabbage above 0.4 a_w were most likely the result of the sorption behavior of fructose and glucose.

The thermograms for cabbage are shown in Fig. 2. As expected, the glass transition temperature depended on the water content, decreasing with increasing humidity in the water sorption range. Moisture content of 60.4g H_2O/100g dry matter was sufficiently high for crystallization to take place above the glass transition temperature. A weight loss smaller than the expected 8.9% was found when freeze-dried cabbage was dried in a DSC pan over P_2O_5. This showed that water was adsorbed when the sample was removed from the desiccator. Calculation of the difference between expected water content and the actual weight loss indicated a water content of 4.5 g/100g d.m.

The transition temperatures and the amounts of freezable and unfrozen water are shown in Table 1. The onset of glass transition was at about room temperature, when water content was 5.2g H_2O/100g dry matter. This is seen in the adsorption isotherms as a rapid increase in the water content (Fig. 1). Freezable water was first observed at a water content of 36.2g H_2O/100g dry matter (Table 1). In the adsorption isotherm this corresponds to an a_w value of 0.68 (Fig. 1). This result correlated well with the sorption measurement, where the last

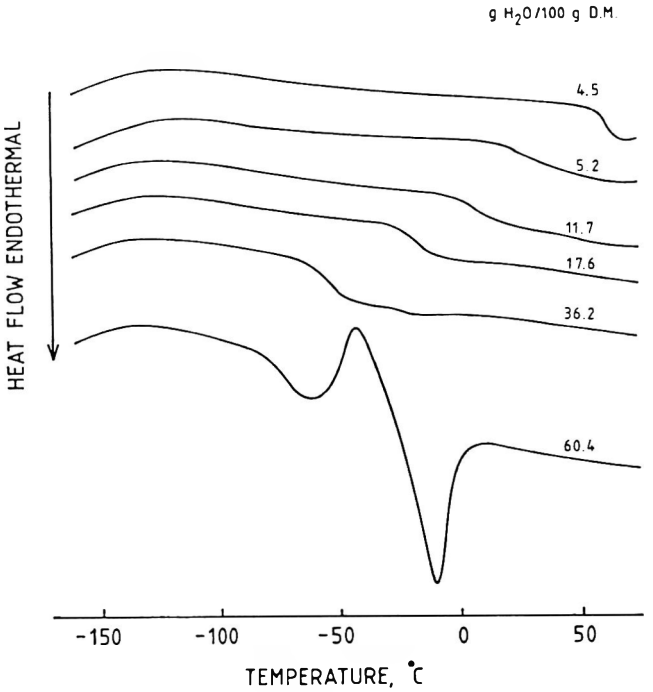

Fig. 2.—DSC thermograms of freeze-dried cabbage. The figures on the curves denote water contents. The energy axis is equivalent in each case, but shifts in each successive curve.

Table 1—Glass transition temperatures and amounts of freezable and unfrozen water in adsorption of freeze-dried cabbage as determined by DSC

Water content g/100g d.m.	Freezable water g/100g d.m.	Unfrozen water g/100g d.m.	Glass transition Onset °C	Glass transition End °C
4.5			38.6	72.7
5.2			10.7	31.1
11.7			−9.2	8.4
17.6			−31.0	−11.3
33.5			−58.4	−44.2
36.2	0.45	35.7	−66.3	−47.5
42.8	1.3	41.5	−68.4	−54.8
60.4	16.8	43.6	−82.9	−69.5
168.8	126.9	41.9		
173.0	133.5	39.5		

measured adsorption equilibrium point was 39.2g H_2O/100g dry matter at 0.69 a_w (23°C). Clearly, in the capillary condensation range solutions of low molecular weight components of the freeze-dried material could affect the freezing behavior. The solvation changes the freezing temperature and capacity, whereas at very high water contents, dilution may promote freezing. The freezable water content of freeze-dried cabbage was very low up to 42.8g H_2O/100g dry matter; however, it increased rapidly above 60.4g H_2O/100g dry matter, while simultaneously the unfrozen water content decreased (Table 1).

CONCLUSIONS

EFFECT of free sugars on the water capacity in cabbage was clearly visible in the sorption isotherms; similarly, the thermograms showed glass transitions. In the water adsorption range, the sorption isotherm was considered to be the main criterion for water content in freeze-dried cabbage. Free water can also be indicated in the thermograms, as the melting of ice. In the capillary condensation range molar solutions of the freeze-dried cabbage affect its unfrozen water content.

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content in terms of human intake, it may be prudent to consider the values on a wet or "as is" basis.

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Inhibition of Undesirable Gas Production in Tofu

CLAUDE P. CHAMPAGNE, BERNARD AUROUZE, and GILLES GOULET

ABSTRACT

Of 100 gas-producing bacterial isolates, 65% were Gram-negative. Bacterial growth and gas production in commercial tofu was accompanied by acidification, and pH decreases as low as 5.2. Coliform bacteria constituted 15% of the total bacterial flora. Sporeformers and yeast counts remained $<10^3$ CFU/mL. Pasteurization was effective in lowering the bacterial counts, and retarded gas production. The addition of lactic acid (reducing pH to 5.5) or lactic acid bacteria (2×10^7 CFU/g) to tofu helped reduce gas production by about 50%. Addition of both lactic acid and *Lactobacillus plantarum* stopped gas production in contaminated tofu.

Key Words: tofu, bacteria, coliforms, pasteurization, gas production

INTRODUCTION

TOFU is a curd obtained from soy milk. Tofu and related products constitute a $100 million-plus industry (Best, 1990), and are gaining wide acceptance in North-America. In supermarkets, tofu is often found in the fruits and vegetables section, where refrigeration may be inadequate, with storage temperatures above 4°C. Since tofu has a relatively high pH (5.8–6.2) and a water content of about 75%, rapid bacterial growth can occur. In addition to off-flavors, undesirable gas production is a common problem associated with microbial deterioration of tofu, resulting in swelled packages. Studies have been reported on gas production in the rumen or the intestinal tracts in relation to soya-based diets (Rackis et al. 1970a,b; Calloway et al. 1971), but none was found on means to prevent gas production in tofu products during storage.

Addition of lactic acid bacteria (LAB) to foods is often used to take advantage of their well known inhibitory activities against undesirable microorganisms (Northolt, 1984), and of their purported health benefits (Kim, 1988). Additionally, fermentation by lactobacilli may reduce objectionable flavors associated with soy products (Stern et al. 1977). No study has been done on addition of LAB to tofu to antagonize growth of undesirable microorganisms.

The objective of our work was to determine the microbial flora associated with unwanted gas production in tofu. We also determined the effectiveness of pasteurization, pH modification and seeding with LAB in preventing gas production in commercial or laboratory samples.

MATERIALS & METHODS

Test organisms and culture maintenance

The various cultures and maintenance procedures, are listed in Table 1. The *Serratia liquefaciens* strain was isolated from a tofu sample, while the LAB were from the Food Research and Development Centre culture collection.

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Gas analysis

The CO_2 content of the gas found in the commercial packaged products was determined using the method of Champagne and Lange (1990).

Microbiological analysis of tofu

The analyses were performed following the recommended methods of Speck (1984). Culture media were from Difco (Detroit). Coliform bacteria were enumerated on Violet Red Bile Agar. Total bacterial counts were obtained on Plate Count Agar to which 0.04 g/L of bromcresol purple was added, which enabled differential enumeration of acid-producing bacteria. Yeast populations were obtained on Potato Dextrose Agar acidified to pH 3.5. Anaerobic sporeformers were enumerated on Reinforced Clostridial Agar following a 85°C/5min treatment of the sample, and plates were incubated in anaerobic jars (BBL, Mississauga, Ont.).

Growth and isolation of bacteria in tofu

Commercial tofu samples of 454 g were incubated at 4 or 25°C for up to 15 days. Some samples were pasteurized by placing vacuum-packed tofus in a 75°C water bath for 30 min. Following microbiological analysis, 100 colonies were picked from the agar plates and inoculated in sterile commercial soy milk. Strong gas production in the soy milk was detected by adding an inverted Durham tube in the broth. Fourteen Gram negative cultures showing strong gas production and different colony characteristics were further isolated and identified with the API 20E system (API Produits de Laboratoire, Saint-Laurent Qué).

Growth of LAB in tofu milk

Sterile commercial soy milks were inoculated with various lactobacilli and lactococci cultures (Table 2) and incubated at 9 or 30°C for up to 120 hr. The inocula were prepared in MRS broth, adjusted at an optical density of 0.3 at 560 nm, and added at a 0.2% (v/v) level.

Inhibition of gas production in tofu

For laboratory assays, sterile ground tofu was prepared by heating 454g portions of commercial tofu at 121°C for 15 min, and aseptically grinding in a Janke & Kunkel (Staufer, Germany) homogenizer. We added 1 mL of a 10% lactic acid solution (Fisher, Montréal) and/or 1 mL a 5×10^9 CFU/mL *Lactobacillus plantarum* LACTACEL cul-

Table 1 – Culture maintenance conditions at 1% inoculation levels

	Medium	Incubation conditions °C/hr	Transfer frequency (wk)
Lactobacillus rhamnosus NG1	MRS	30, 24	1
Lactobacillus casei NG2	MRS	30, 24	1
Lactobacillus plantarum LACTACEL	MRS	30, 24	1
Lactobacillus plantarum ATCC 1717	MRS	30, 24	1
Lactobacillus plantarum ATCC 14917	MRS	30, 24	1
Lactobacillus plantarum ATCC 1443	MRS	30, 24	1
Lactobacillus plantarum ANO 330	MRS	30, 24	1
Lactococcus lactis CRA-1	Milk[a]	23, 16	0.5
Lactobacillus acidophilus CRDA-200	MRS	37, 24	1
Bifidobacterium bifidum CRDA-600	MRS	37, 24	1
Serratia liquefaciens NG3	SRP[b]	30, 24	2

[a] Milk = nonfat dry milk rehydrated at 12% (w/w) solids; sterilized 121°C/10 min.
[b] SRP = Broth composed of 1% sucrose, 0.5% raffinose and 0.5% soya peptone. CRDA-200 and CRDA-600 isolated from a commercial Nu-Trish product.

Table 2—Fermentation of soya milk by lactic acid bacteria; pH of soya milks. (Initial pH 6.50)

	9°C (120 hr)	30°C (22 hr)
Lactobacillus rhamnosus NG1	6.44	6.29
Lactobacillus casei NG2	6.45	4.92
Lactobacillus plantarum LACTACEL	6.44	5.15
Lactobacillus plantarum 1717	6.38	4.99
Lactobacillus plantarum ATCC 14917	6.39	5.04
Lactobacillus plantarum ATCC 14431	6.44	5.12
Lactobacillus plantarum ANO 330	6.47	6.39
Lactococcus lactis CRA-1	6.39	6.31

ture to 46 g of sterilized ground tofu, and mixed in a Stomacher Lab Blender 400 (London). One sterile tofu sample was used as a negative control, by adding 2 mL of sterile water. The other samples were inoculated with a *Serratia liquefaciens* culture at an initial contamination level of 10^5 CFU/g. Gas production was evaluated by Risograph (Pullman, USA). We found that the facultative *Serratia* had an oxidative metabolism in the ground tofu, which interfered with the gas production evaluation of Risograph. In order to prevent this oxygen consumption by *Serratia*, 40 mL of soya oil was added to all ground tofu mixes, thus generating microaerophilic conditions favorable to a fermentation metabolism (and simulating the microaerophilic conditions encountered in a vacuum-packed product). The samples were incubated at 26°C for up to 40 hr. The lactobacilli and *Serratia* populations were followed by plating on acidified (pH 5.5) MRS agar or VRBA respectively.

Some assays were performed under industrial conditions by dipping 420 g tofu blocks in a 2% lactic acid solution for 6 min, or by adding 1 or 10 mL of a LAB culture (having about 10^9 CFU/mL) in the bag prior to vacuum-packing. The lactococcal cultures were suspended in a 10% glucose solution. The tofu products were incubated at 23°C for 4 days, and gas production caused swelling of the packaging material. Gas production was estimated by the water displacement volume.

Statistical analysis

Results are averages of 3 or more separate trials. Duncan's multiple range variance analysis was performed using SAS software (Cary, NC.).

RESULTS & DISCUSSION

Microbial flora of commercial samples

The CO_2 content of gas samples taken from swollen (average 53%) commercial tofus were between 36 and 85%. We did not determine the composition of the non-CO_2 gas. It is well established that coliform bacteria and clostridia generate H_2 and CO_2 in equal proportions (Doelle, 1975). The 53% CO_2 content suggested that gas production was not limited to heterofermentative lactobacilli, yeasts or propionibacteria which mainly produce CO_2 as a gas metabolite. Probably the main gas-producing organisms were coliform or clostridia.

When stored at 25°C, the total bacterial population of commercial tofu samples could reach 10^9 CFU/g in only 24 hr (Fig. 1). The initial populations of these samples were abnormally high (10^6 CFU/g), since freshly produced samples generally showed bacterial counts between 10 and 1000 CFU/g. Yeast counts remained below 10^3 CFU/g in all samples. Two commercial tofu products were analyzed: traditional and onion-pepper containing tofus. Sporeformers were not found in traditional tofu, but reached 10^3 CFU/g in onion-pepper containing products of the various floras analyzed, which suggested that the spores were from the condiments. The sporeformers were the only population to be significantly ($P = 0.01$) influenced by addition of condiments. The total coliform population (at 25°C) was significantly ($P = 0.03$) lower than the total bacterial counts (15% of total bacterial floras) of deteriorated tofus. Not all species that grew in tofu were gas producers. Of the 100 colonies picked from the PCA or VRBA agar plates, about 60% of the cultures produced gas in soya milk. Most isolates were Gram negative (65%). Using the API 20E system, we attempted to identify 14 Gram negative strains, and found that the largest group were of the genus *Serratia* (Table 3). These results, combined with gas analyses, suggested that enterobacteriaceae, which produce H_2 in addition to CO_2 (Doelle, 1975), could have an important part in undesirable gas production although they were a minor fraction of total bacteria.

Storage at 4°C slowed bacterial development, and the tofus did not show evidence of gas formation following 15 day incubation though the total bacterial population had reached 10^8 CFU/g (Fig. 1). If stored at high temperatures, tofu often develops undesirable gas production. Soya may cause flatulence in the digestive system as a result of the fermentation of stachyose, raffinose and sucrose by intestinal flora (Rackis et al., 1970b). Not all microorganisms can assimilate those sugars, and specific bacterial flora may develop. In studies on the effects of a raffinose rich diet on intestinal flora, the bifidobacteria, the lactobacilli and the enterobacteriaceae populations increased while clostridial flora proportionally tended to decrease (Benno et al., 1987). These observations seemed to extend to tofu since the most frequently isolated Gram negative gas-producing species in swollen tofu products was an enterobacteriaceae (*Serratia*), the acid-producing population in tofu increased during storage (see Acidification section), and sporeformers were not important.

Pasteurization of commercial tofus

The tofu curd was obtained at 90°C and, following a 420g block formation, was cooled in water and packed. Surface contamination of the tofu blocks was thus possible through contact with water, conveyor belts and packaging materials. A post-wrapping heat treatment was applied to help eliminate surface contamination. Pasteurization of commercial tofus reduced initial counts by a factor of 10000 (Fig. 2). The total

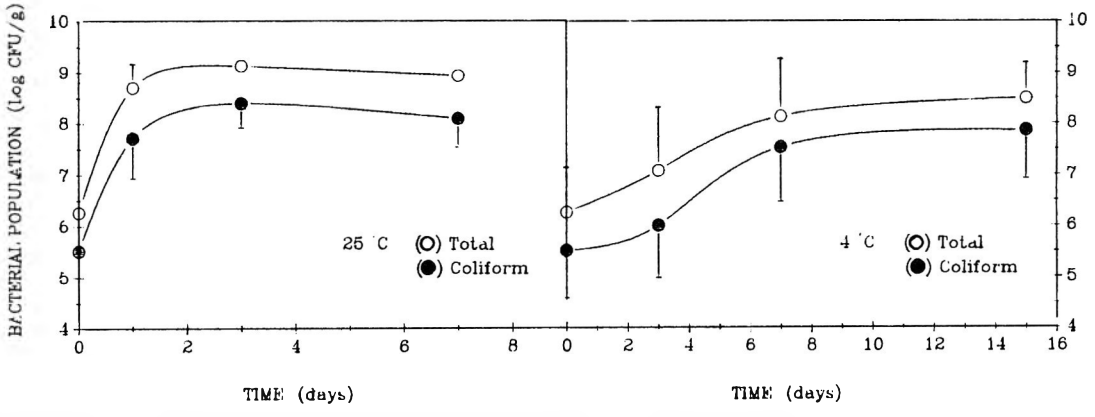

Fig. 1—Bacterial growth in untreated tofu stored at 4 or 25°C. Bars are 95% confidence limits.

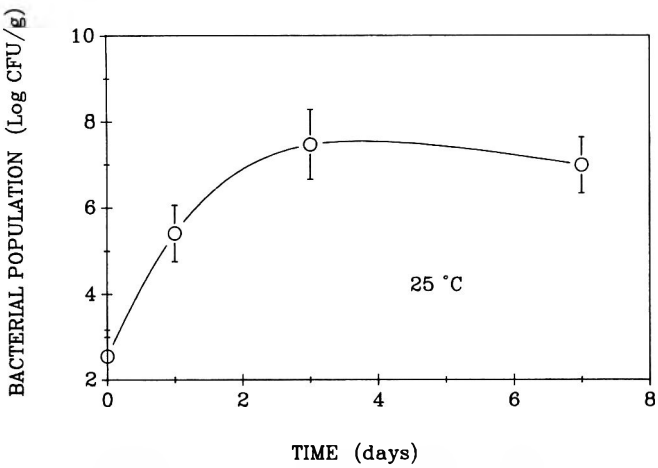

Fig. 2—Bacterial growth in pasteurized tofu at 25°C. Bars are 95% confidence limits.

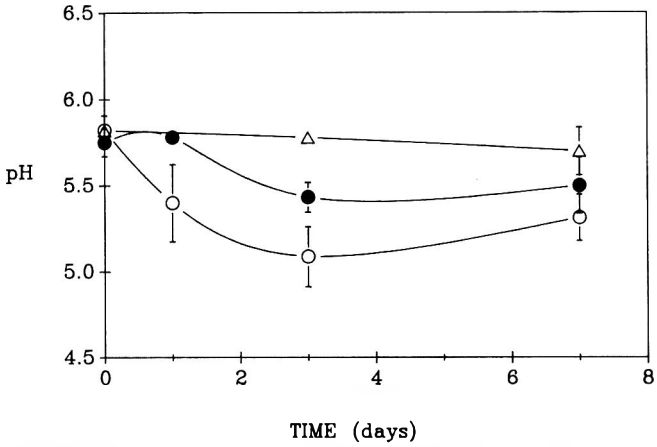

Fig. 3—Changes in tofu pH during storage. Bars are 95% confidence limits. (○) untreated tofu, 25°C; (●) pasteurized tofu, 25°C (△) untreated tofu, 4°C.

Table 3—Gram negative, gas-producing bacteria isolated from deteriorated tofu[a]

Species	Isolates
Serratia liquefaciens	9
Serratia sp.	1
Escherichia coli	1
Enterobacter sp.	2
Klebsiella ozonae	1

[a] 100 colonies were picked from 4 tofu samples. Of the 21 Gram negative, gas-producing strains, 14 were identified with API 20E.

bacterial population at the stationary growth phase was significantly lower ($P \leq 0.04$) when the sample had previously been pasteurized. We did not determine the reason for this. That the pasteurization treatment destroyed heat-labile growth factors was unlikely since the temperature (75°C) was lower than that to soak the soy beans and prepare the soy milk (90°C). Thus heat-labile growth factors were probably destroyed well before pasteurization of samples. Gas production occurred upon storage (25°C) of the pasteurized tofus, though the coliform counts remained below 10^3 CFU/g. In addition to partial elimination of the contamination population, pasteurization modified the bacterial make-up of the tofu. With the pasteurized products, packages could be stored twice as long before swelling, as for the untreated samples. Pasteurization was thus effective in retarding undesirable production of gas under storage at 25°C, but not in preventing it.

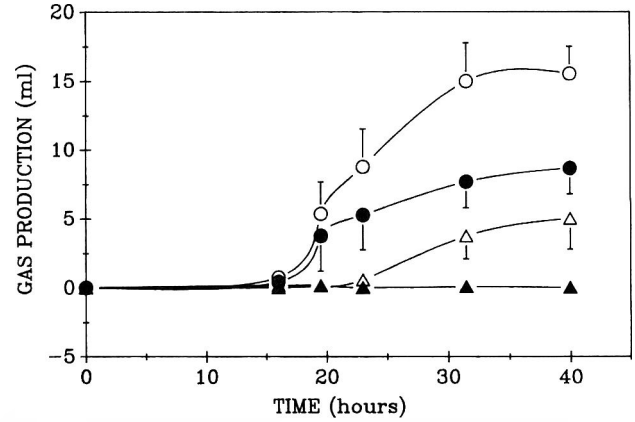

Fig. 4—Effect of the addition of lactic acid and/or Lactobacillus plantarum *on gas production in tofu contaminated with* Serratia liquefaciens. *Bars are 95% confidence limits. (○)* Serratia *control; (●)* Lactobacillus *added; (△) Lactic acid added; (▲)* Lactobacillus *and lactic acid added.*

Acidification of commercial tofus

Bacterial growth was accompanied by acidification of tofus (Fig. 3). The acid-producing bacteria represented 10% of the flora on day 1 and 3 but constituted about 50% on day 7. This increase in % acid producing organisms from day 3 to day 7 was accompanied by a slight increase in pH of samples stored at 25°C (Fig. 3). As observed for growth and gas production, acidification of pasteurized samples occurred later during incubation, and was significantly lower than for untreated tofus (Fig. 3).

Addition of lactic acid or LAB

The purpose of seeding tofu with LAB is that they would only proliferate in the event of temperature abuse of the product. Thus a well stored tofu (2°C) would not develop an acid taste. Under temperature abuse an acid taste would develop, but this seems preferable to the unclean flavor associated with coliform flora. For our laboratory assays we selected a strain that grew well in soy milk at room temperature, but which demonstrated little acidifying activity at refrigerated temperatures. Most *Lactobacillus plantarum* cultures grew well in soymilk, which confirmed results of Angeles and Marth (1971), and strain Lactacel was chosen for our laboratory assays since it demonstrated little activity at 9°C (Table 2).

In laboratory assays, only the combined addition of lactic acid and *Lactobacillus plantarum* was effective in preventing gas production (Fig. 4). Growth of the lactobacilli was slightly inferior in the sample to which lactic acid was also added, but development of the *Serratia* culture was 20 times less in this mix as compared to the positive control (Fig. 5). The final pH was the lowest in the samples to which lactic acid and *Lactobacillus plantarum* had been added (Fig. 6). Seeding with *L. plantarum* was not as effective, in the first 20 hr in preventing gas production as was direct addition of lactic acid (Fig. 4). This was probably related to the lag time required for growth and acidification of *L. plantarum*. In this respect, effectiveness of *L. plantarum* was probably related to the level of contamination with undesirable bacteria. Our studies were performed under relatively high contamination levels (10^5 *Serratia* CFU/g). We assumed that, under temperature abuse, the time required for the seeded LAB to acidify the tofu would be relatively constant. On the other hand, the time required for contamination flora to reach the level required for defects to appear would depend on initial contamination level. Thus, with initial low bacterial contamination, the gas-producing population may not reach the level for defects since an inhibitory pH range would be attained before that level could be reached.

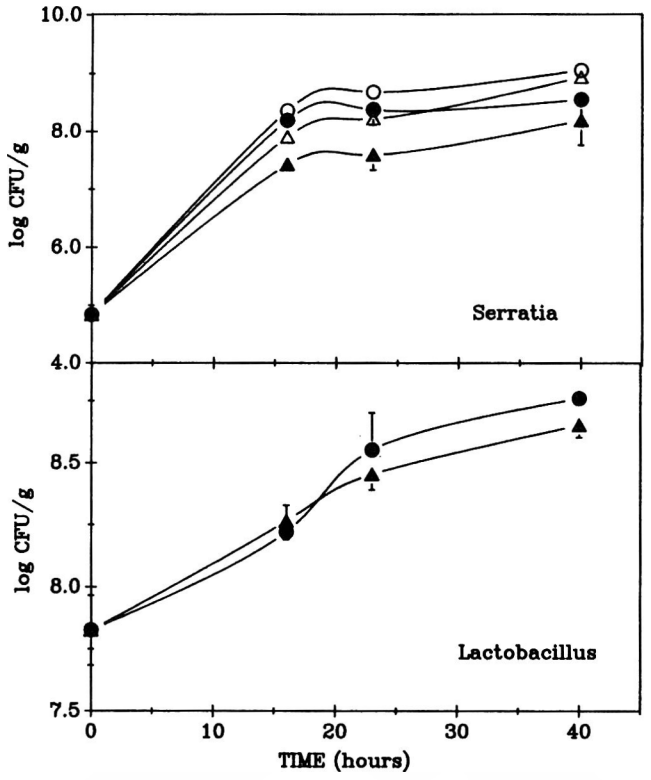

Fig. 5—Growth of Serratia *(top panel) or* Lactobacillus plantarum *(bottom panel) in tofu.* Serratia *was added to all samples while* L. plantarum *was added to two samples. (○)* Serratia *control; (●)* Lactobacillus *added; (△) Lactic acid added; (▲)* Lactobacillus *and lactic acid added.*

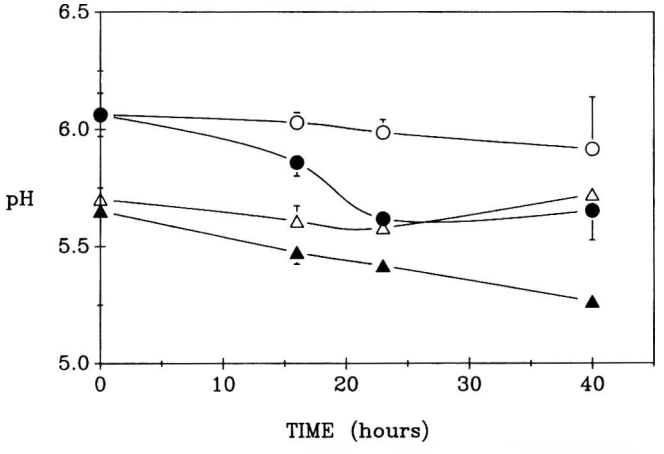

Fig. 6—Change in tofu pH following addition of Serratia, *lactic acid and/or* Lactobacillus. *Bars are 95% confidence limits. (○)* Serratia *control; (●)* Lactobacillus *added; (△) Lactic acid added; (▲)* Lactobacillus *and lactic acid added.*

With industrial assays, we found that dipping of tofu blocks in a 2% lactic acid solution lowered pH from 5.78 to 5.29. This had a notable effect on gas production (Table 4). As was found in the laboratory assays, addition of lactic acid or LAB alone, were insufficient to prevent gas production at 25°C. Addition of thermophilic strains (*Bifidobacterium, Lactobacillus acidophilus*) provided a safeguard against overacidification

Table 4—Effect of dipping tofu blocks in 2% lactic acid or addition of lactic acid bacteria (LAB)[a] cultures on gas production in commercial 454g tofu blocks[b]

Treatment	Gas produced (mL)
Control	136
Lactic acid	75
Thermophilic LAB (2×10^6/g)	105
Thermophilic LAB (2×10^7/g)	49
Lactococcus lactis CRA-1 (10^6/g)	67
Lactococcus lactis CRA-1 (10^7/g)	30

[a] Thermophilic LAB were an equal mixture of *Lactobacillus acidophilus* and *Bifidobacterium bifidum.*

[b] 6 days incubation at 25°C

during storage at 4°C, but they were not as effective as the mesophilic strain. In these assays we also tested whether cultures that could not acidify soymilk could be used for inhibition of gas-producing organisms. The culture added need not be well adapted to tofu carbohydrates to be effective. Our results showed that *Lactococcus lactis* did not acidify well soymilk alone (Table 2), but was effective in lowering gas production if the bacterial suspension was prepared in 10% glucose. The potential disadvantage of this approach is that addition of glucose may also stimulate growth of contaminating flora. The industrial testing of lactic acid addition showed there were important variations in effectiveness of this approach. Heat treatment of the packed tofus seemed to better control undesirable gas production. Pasteurization also did not modify taste of the product. Addition of lactic acid to pH 5.2 to 5.4 resulted in a noticeably acid taste. Therefore, although pasteurization was not as effective as combined lactic acid/LAB addition in controlling undesirable gas production, it appeared to be the most practical solution under industrial conditions.

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Calcium Sulfate Concentration Influence on Yield and Quality of Tofu From Five Soybean Varieties

NONG SUN and WILLIAM M. BREENE

ABSTRACT

A mini-process is described for making tofu from 50-g quantities of soybeans. It was used to compare yield and quality among 5 Minnesota grown varieties (Vinton, Corsoy, Hardin, Stine 2510, Stine 2810) and to determine optimum concentration of the coagulant, $CaSO_4$. Product yield (wet basis), solids recovery, protein recovery and textural quality (Texture Profile Analysis hardness and fracturability) were optimal at 0.02 N $CaSO_4$ for all 5 varieties. Negative correlations were found between $CaSO_4$ concentration and both yield ($r = -0.90$ to 1.00) and protein recovery ($r = -0.96$ to 1.00) for all varieties.

Key Words: tofu-quality, soybean, coagulants, tofu-yield, calcium sulfate

INTRODUCTION

SOYBEAN VARIETY has been shown to influence yield and quality of tofu (Skurray et al., 1980; Kamel and De Man, 1982; Wang et al., 1983; Lim et al., 1990) due to variations in composition, e.g., protein, oil, ash and phosphorus content, among others. However, in order to make valid comparisons among varieties, it is important to produce tofu by a standardized procedure because manufacturing variables can profoundly influence yield and quality. These variables include time and temperature of presoaking soybeans prior to making soymilk (Watanabe et al., 1964; Shurtleff and Aoyagi, 1979; Hsu, 1983; Hsu et al., 1983), the water: soybean ratio in the soymilk (Shurtleff and Aoyagi, 1979; Beddows and Wong, 1987a), time and temperature of heating the soymilk (Watanabe et al., 1964; Saio, 1979; Shurtleff and Aoyagi, 1979) temperature and extent of stirring during coagulation (Wolf and Tamura, 1969; Wang and Hesseltine, 1982; Beddows and Wong, 1987b) and type and concentration of coagulant (Wantanabe et al., 1964; Appurao and Narasinga Rao, 1975; Saio, 1979; Shurtleff and Aoyagi, 1979; Skurray et al., 1980; Tsai et al., 1981; Wang and Hesseltine, 1982; Wang, 1984; Yasuda and Hokama, 1984; De Man et al., 1986; Beddows and Wong, 1987c).

The coagulant of choice among most tofu makers is calcium sulfate; Tsai et al. (1981) and Wang and Hesseltine (1982) found it superior to other calcium salts. Nigari, a by-product of the maufacture of sea salt, consists mainly of magnesium sulfate, but can vary in composition and has not been approved for use in the U.S. (Shurtleff and Aoyagi, 1979). Glucono delta lactone (GDL) is used to make soft (silken) tofu, but is not suitable for making firm (Chinese style) tofu if used alone (Tsai et al., 1981).

The objective of our study was to determine how yield and quality might be influenced by the concentration of coagulant, calcium sulfate, in tofu made from five different Minnesota grown soybean varieties using a small-scale procedure to mimic industrial practices.

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MATERIALS & METHODS

Materials

Five Minnesota-grown, light-hilum soybean varieties from the 1987 harvest were obtained from North Country Seed, Inc., Trimont, MN. They were selected to include the popular choice of many tofu makers (Vinton), two varieties very popular among growers (Corsoy and Hardin) and two newer introductions (Stine 2510 and Stine 2810).

All chemicals used in the analyses were reagent grade and were obtained from Sigma Chemical Co., St. Louis, MO.

Hydration rate

Fourteen 50-g samples of soybeans of each variety were immersed in distilled 22°C water in separate beakers. After soaking at 22°C for 2, 4, 6, 8, 10, 12 or 14 hr, duplicate samples were drained on a screen for 30 sec and weighed to determine the amount (g) of water absorbed.

Soymilk and solids content

Washed soybeans (50 g) were soaked in 500 mL beakers at 22°C for 12 hr in about 400 mL tap water, drained, rinsed once with 400 mL tap water, combined with 500 mL tap water and ground for 2 min in an Osterizer seven-speed blender at "liquefy" speed. Five drops of Antifoam A emulsion (Dow Corning Corp., Midland, MI) were added and the slurry was boiled in an aluminum saucepan for 15 min and immediately filtered through a muslin cloth (one thickness) to remove the okara.

Soymilk solids content was determined using an Abbe refractometer (American Optical Model 10450) and a standard curve in which refractive index (Y axis) was plotted vs oven dry solids content (X axis) of 1-mL soymilk samples in the solids range 4 to 11%. Refractive index was determined at room temperature and correlated highly with oven dry moisture results. This quick method was recommended for industry use by Johnson and Wilson (1984).

Preparation of tofu

A 325-mL portion of hot soymilk was poured into 40 mL of Food Grade calcium sulfate ($CaSO_4 \cdot 2H_2O$, Van Waters & Rogers, Inc., Seattle, WA) solution of the appropriate concentration in a 500 mL beaker. For final concentrations in soymilk of 0.01, 0.02, 0.03, 0.05, and 0.06N $CaSO_4$, this required 0.63, 1.26, 1.89, 3.15, and 3.78g $CaSO_4$/40mL, respectively. The 365 mL of soymilk/coagulant was held at 70°C for 10 min in a water bath while coagulation occurred. The soybean curd was then transferred, taking care to avoid breakage, to a specially designed 7.5 × 7.5 × 7.5 cm cheesecloth-lined polymethyl methacrylate form and pressed for 2 hr by placing a 560g weight on the 56.25 cm^2 plate covering the curd. The six-sided form could be easily assembled and disassembled for cleaning.

Tofu yield, protein and solids recovery, and proximate analyses

Yield is expressed as wet weight of tofu obtained from 50g of soybeans. Protein recovery is expressed as the amount of protein in the tofu dry matter divided by the amount of protein in the soybean dry matter times 100. Solids (dry matter of tofu) recovery was determined by the oven dry method.

About 1 kg of soybeans of each variety was ground in a Thomas-Wiley Mill to pass a 1-mm diameter mesh sieve using liquid nitrogen to prevent moisture loss and protein denaturation and to keep the oil in the solid state.

Samples were analyzed for moisture (Williams and Baker, 1984, AOAC 14.081, 14.004); oil (Link, 1973, AOCS AC 3-44); ash (Wil-

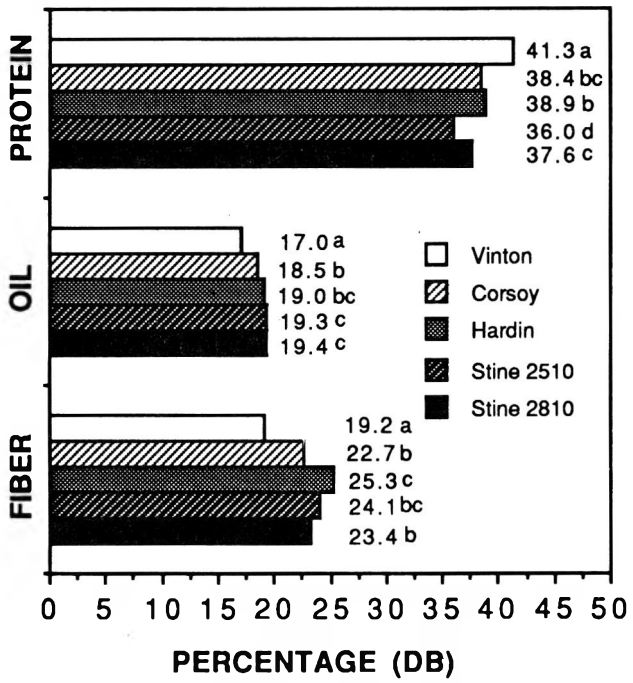

Fig. 1—Dry basis (DB) protein, oil and neutral detergent fiber contents of five soybean varieties. Means of duplicates; different letters adjacent to values denote differences (P<0.05).

liams and Baker, 1984, AOAC 14.082, 7.009) and crude protein (Williams and Baker, 1984, AOAC 2.057, modified Kjeldahl method).

Neutral detergent fiber (NDF

Neutral detergent fiber (NDF) was determined by the modified method of Van Soest and Wine (1967). Duplicate 1.00±0.10g samples of soybeans or tofu were weighed in tared 30 mL Pyrex glass filtering crucibles; 100 mL of neutral detergent solution was added. The crucibles were placed in a Tecator Fibertec System M 1020 Hot Extractor and samples were boiled for 1 h. The digested samples were rinsed well several times with hot tap water. Residues were rinsed again twice with acetone and dried overnight in a 100–110°C oven.

Mineral content

Ground soybean samples containing about 1g of dry matter were accurately (±0.001g) weighed into 20-mL high-form silica crucibles and dry-ashed in a muffle furnace at 485°C for 10–12 hr. Crucibles were covered during ashing. Ash samples were each equilibrated with 10 mL 2N HCl at room temperature for 3 hr followed by transfer of the supernatant to a 7 mL plastic disposable tube for simultaneous direct analysis of Ca, Mg, Na, K, P, Fe, Mn, Al, Cu, Zn, Cd, Cr, Ni, Pb and B by Inductively Coupled Plasma (ICP) Atomic Emission Spectrometry.

Textural properties and color

The Texture Profile Analysis (Szczesniak, 1975) parameters of hardness and fracturability were determined on 2.0 cm diameter by 2.0 cm high cylindrical samples on a Model 1122 Instron Universal Testing Machine (0-2 kg load cell capacity). Samples were taken such that compression force was exerted on the same plane as the compression force during tofu pressing. The samples were compressed from 2 to 0.5 cm (75% deformation). Crosshead and chart speeds were 20 and 50 mm/min, respectively. Nine replicate samples were tested for each 50-g soybean batch of tofu.

A Minolta Chroma Meter CR-200 was used for color reflectance determination. One determination was made for each variety of soybeans or each batch (50 g soybeans) of tofu.

Statistical analysis

Data were analyzed by ANOVA using the Statistical Analysis Systems program (SAS, 1985). Means comparisons were made by Least Significance Differences (LSD) procedure ($p<0.05$).

RESULTS & DISCUSSION

Analyses of soybeans

Vinton had the highest protein and the lowest oil content while Stine 2510 had the lowest protein content but a higher oil content that was the same as those of Stine 2810 and Hardin (Fig. 1). Vinton had the lowest and Hardin the highest NDF content. Negative relationships existed between protein and fiber ($r = -0.71$) and protein and oil content ($r = -0.87$). This was in agreement with Caviness (1973) and Wang et al. (1983) who found that protein and oil contents of soybeans correlated negatively.

Moisture, size and color data are listed in Table 1. Size was expressed in terms of the weight (g) of 100 soybeans. Seeds of Vinton were the largest and Corsoy the smallest. L values showed Vinton, Corsoy and Hardin to be lighter-colored than Stine 2510 and 2810. Redness (a) ranged from 2.7 in Hardin to 5.1 in Stine 2510; yellowness (b) ranged from 25.8 to Vinton to 33.6 in Hardin. All five varieties had yellow hila. Soybeans with light hila are preferred for tofu making (Wang et al., 1983).

The total ash contents of the five varieties were essentially the same (Table 1). The main differences among individual minerals that might relate to tofu yield and quality were higher contents of Ca in Vintron and lower Mg in Stine 2510. A strong linear relationship existed between protein and Ca contents. P content varied somewhat and K content was similar among varieties. The contents of trace minerals were also similar (data not shown). Their contribution to tofu yield would be minor and their main contribution to tofu quality would be nutritional.

Hydration of soybeans

To make tofu, soybeans are presoaked to soften their cellular structure, reduce the amount of energy required to grind them, and increase the subsequent rate of nutrient extraction. The hydration rate depends on the temperature of the soaking water and the variety and age of the soybeans; the colder the water, the slower the hydration (Shurtleff and Aoyagi, 1979; Hsu et al., 1983). In our studies, hydration occurred rapidly and similarly in all five soybean varieties up to 4 hr after which the rates leveled off. Complete hydration required about 12 hr, which was similar to results reported by Hsu et al. (1983). All varieties had similar hydration rates which appeared to be normal. Therefore, a 12-hr soak was employed in our studies. Typical soaking times at ambient temperature can vary from

Table 1—Moisture, size, color (Hunter L) and proximate analyses of five soybean varieties[a]

Variety	Moisture %	g/100 beans	L	% Ash	Ca	Mg	P	K
					mg/100 g			
Vinton	10.4	23.0	65.6	5.3	269	307	644	1976
Corsoy	11.8	16.2	66.0	5.2	250	252	493	1953
Hardin	11.6	18.2	65.8	5.3	254	259	530	2010
Stine 2510	10.5	18.0	59.5	5.3	237	256	543	2039
Stine 2810	10.5	17.1	57.8	5.3	256	292	571	2018

[a] Means of duplicates.

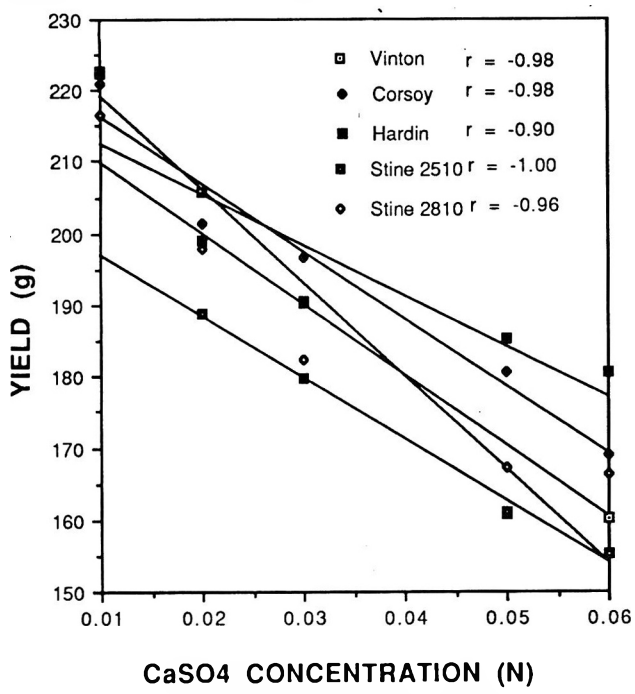

Fig. 2—Effect of soybean variety and $CaSO_4$ concentration in soymilk on yield of fresh tofu from 50g of soybeans.

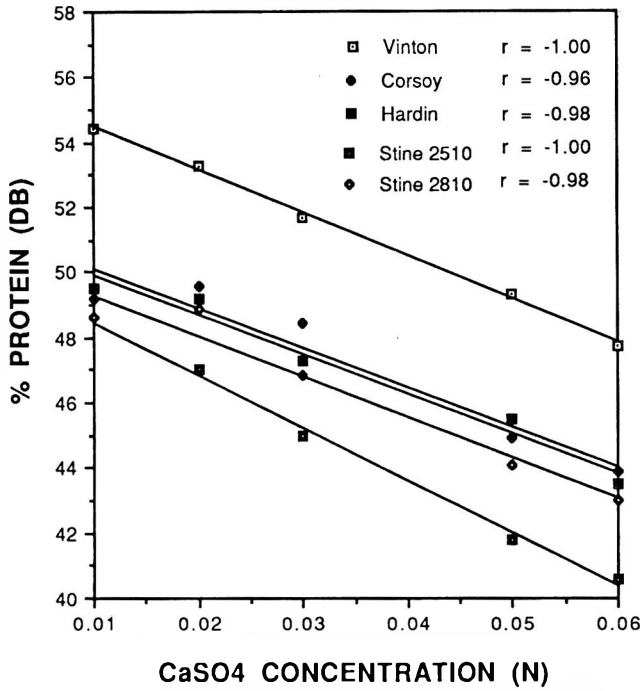

Fig. 3—Effect of soybean variety and $CaSO_4$ concentration in soymilk on protein content (dry basis) of tofu from 50g of soybeans.

8 to 10 hr in summer to 10 to 20 hr in winter in Japan; hydration rates differ among varieties (Watanabe et al., 1964). Soybeans that are small and hard, older than 6 mo and/or high in oil may have impaired hydration. Too much or too little moisture uptake by beans prior to soymilk extraction can affect quality and yield of tofu (Shurtleff and Aoyagi, 1979).

$CaSO_4$ concentration effects on tofu yield and quality.

Yield and quality of tofu can be affected by concentration of the coagulant, $CaSO_4$. The effects of soybean variety and

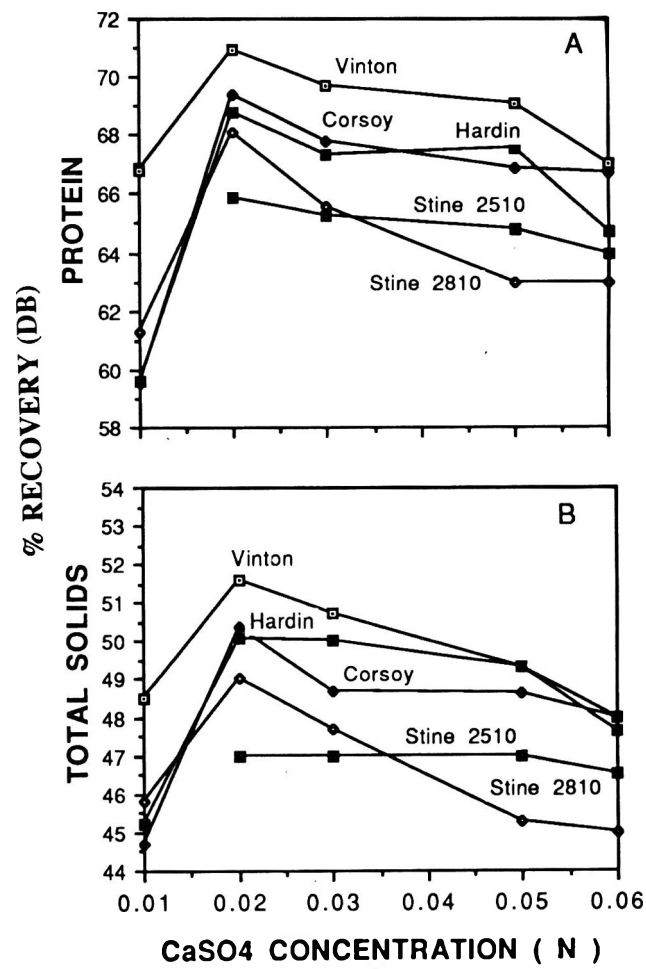

Fig. 4—Effect of soybean variety and $CaSO_4$ concentration in soymilk on percentage recovery of protein (A) and total solids (B) in tofu.

$CaSO_4$ concentration on yield, percent protein in tofu, total solids recovery, protein recovery, fracturability and hardness of tofu are shown in Fig. 2 through 5. Negative linear regression relationships were found between $CaSO_4$ concentration and both yield (Fig 2) and percent protein (Fig 3) in tofu. The decrease in yield with increasing $CaSO_4$ (calcium ion) concentration could be due to increasing syneresis and loss of whey from the curd as more bonding occurred thus making the protein matrix more dense and compacted. The regression lines were roughly parallel for tofu yield from Corsoy, Stine 2810 and Stine 2510 compared to a lesser decrease in yield with increasing $CaSO_4$ concentration for Hardin and a greater corresponding decrease for Vinton. Thus, Vinton was the most sensitive to changes in $CaSO_4$ concentration.

The percent protein in tofu (DB) showed parallel decreases with increased coagulant concentration for all five varieties (Fig 3) indicating that increased syneresis resulted in loss of soluble protein (whey protein) along with water. The data also reflect overall differences in protein content of the five soybean varieties, decreasing in the order Vinton, Corsoy, Hardin, Stine 2810, Stine 2510. Although increasing $CaSO_4$ concentration produced the most dramatic decrease in yield for Vinton, a corresponding decrease in protein content was not observed. Therefore, yield reduction must have been due primarily to loss of water through syneresis.

Figure 4 shows maximum recovery of both protein and total solids in tofu at 0.02N $CaSO_4$, with recoveries much lower at 0.01N $CaSO_4$ and a gradual decrease from 0.02N to 0.06N $CaSO_4$. At the lower concentration, bridging of protein molecules by calcium ion is not sufficient to form a firm gel. At

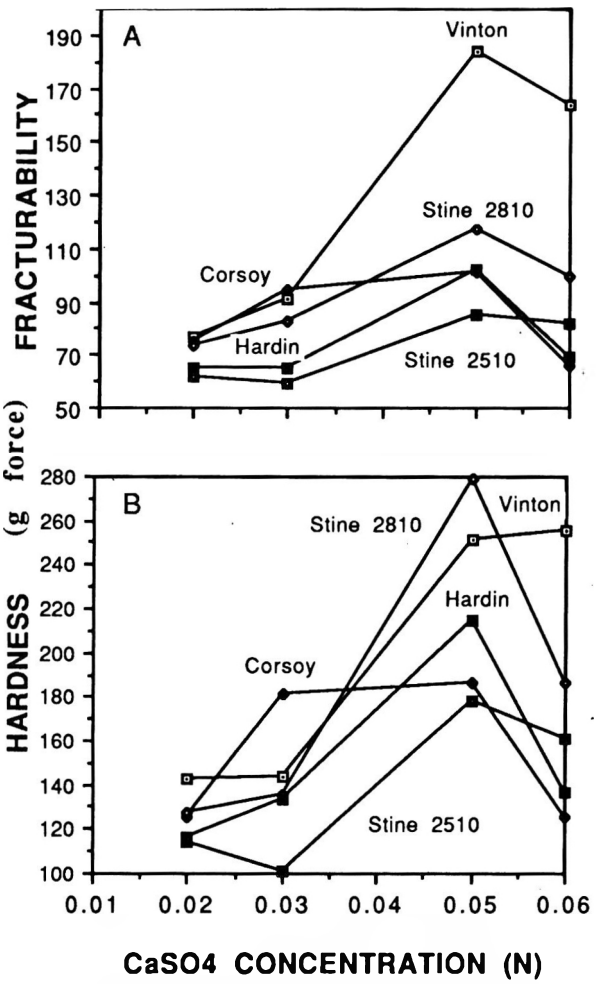

Fig. 5—Effect of soybean variety and $CaSO_4$ concentration in soymilk on fracturability (A) and hardness (B) of tofu.

higher concentrations, increased bridging and the resulting compaction of the protein matrix cause increased syneresis and loss of water, whey protein and other solubles. Although the highest tofu yields were obtained at 0.01N $CaSO_4$ (Fig. 2), this was due mainly to a high moisture content in the pressed product. These tofu products did not assume a distinct cubical form and did not retain their shape after cutting. At 0.02N $CaSO_4$, the tofu had an even texture and good retention of cut shape.

Figure 5 depicts the effects of $CaSO_4$ concentration on TPA fracturability and hardness. Tofu made with 0.01N $CaSO_4$ was too soft to enable sampling for the TPA procedure. As coagulant concentrations increased above 0.02N $CaSO_4$, the trend was toward an increase in fracturability and hardness up to 0.05N $CaSO_4$ and then a decrease at 0.06N $CaSO_4$. Vinton tofu showed the greatest increase in fracturability with increasing $CaSO_4$ concentration and considerably higher fracturability than the others at the 0.05N and 0.06N levels (Fig. 5A). Vinton tofu recovered the highest percentage of total solids and protein at 0.05N $CaSO_4$, whereas Stine 2810 tofu recovered the least (Fig. 4). They were similar in hardness at that coagulant level and both were harder than the other 3 (Fig. 5B). These tofus were all sorfter than those described by Wilson et al. (1983) who used a different coagulation temperature. Increasing the $CaSO_4$ concentration to 0.06N produced little, if any, change in hardness of Vinton tofu (Figure 5B), but a dramatic decrease in that of Stine 2810 tofu. This suggested that soybean solids other than protein may play a role in tofu textural quality. This could be through interactions between carbohydrates and proteins as hypothesized by Lin et al. (1990).

No formal sensory texture evaluations were made. One author (NS) is Chinese and a lifelong consumer of tofu, noted that tofu texture was best and quite acceptable at 0.02N $CaSO_4$. Also, as $CaSO_4$ concentration was increased above 0.02N, the tofu products became harder, coarse and rubbery and had the appearance of precipitates rather than gels. Miura and Komeyasu (1981) found sensory hardness correlated highly with instrumental hardness, so it appears that instrumental hardness is a valid and useful parameter for quality evaluation of tofu. These observations that addition of $CaSO_4$ to soymilk at 0.02N produced optimum yields and textural quality of tofu were in agreement with previous reports (Saio, 1979; Yasuda and Hokama, 1984).

CONCLUSIONS

A LAB SCALE mini-procedure could differentiate between soybean varieties for relative potential for tofu making. However, additional work is needed to verify that this method could predict the behavior of different soybean varieties and processing variables under large scale tofu processing conditions. The concentration of the coagulant $CaSO_4$ in the soymilk affects the yield, protein and solids recoveries and textural quality of tofu from a given quantity of soybeans. These were optimum at a $CaSO_4$ concentration of 0.02N in soymilk from 5 different varieties of Minnesota-grown soybeans. For screening varieties, we recommend the mini-procedure described here and a $CaSO_4$ concentration of 0.02N in the soymilk.

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Breadfruit Chips: Preparation, Stability and Acceptability

R. P. BATES, H. D. GRAHAM, R. F. MATTHEWS and L. R. CLOS

ABSTRACT

Mature breadfruit, sliced to 1.25 mm, fried in soy oil at 165°C and salted to 1.5% produced a stable, crisp chip with lipid stability comparable to potato chips. Partial air drying prior to frying reduced oil absorption from 42% to 26%. Sensory evaluations showed the product to be as acceptable as commercial plantain or potato chips. In view of the extreme perishability and high postharvest losses of breadfruit, chip production represents a useful value-adding preservation method meriting consideration in breadfruit producing regions.

Key Words: breadfruit, fried-chips, acceptability, storage-changes, lipids, peroxides

INTRODUCTION

BREADFRUIT is important in the diet of people of the tropics. It is relatively cheap and is easily available during its seasons (normally two crops/year are produced). Its nutritional value has been established by Graham and Negron (1981). Breadfruit is a good source of carbohydrates (Graham and Negron, 1981; Wooton and Tumaalii, 1984). Compared with the banana, cassava, plantain, taro, and sweet potato, it is a relatively good source of calcium (Monroe et al., 1986). Potassium and phosphorus have been reported in relatively good quantities although amounts may vary between cultivars (Graham and Negron, 1981; Wooton and Tumaalii, 1984, Munroe et al., 1986). When compared to other tropical starchy foods, it is an acceptable source of vitamin C (20 mg/100 mg of pulp) and has good levels of iron, niacin and riboflavin at all stages of maturity (Graham and Negron, 1981). Though not high in protein, the amino acid profile of its protein was found to be favorable (Patel and Graham, unpublished data; Nochura, 1985; Arcelay and Graham, 1980).

The main problem with breadfruit utilization is its high perishability, resulting in high postharvest losses. In some extreme cases up to 50% losses have been reported (Coursey et al., 1976). This is due mainly to the high postharvest rate of respiration (Wooton and Tumaalii, 1984; Marriot et al., 1979). Consequently, only fruits for immediate needs are harvested, thus reducing the opportunities for development of a large scale international trade in breadfruit.

The breadfruits are round, oblong or oval in shape, with green skin composed of polygonal sectors. Mature fruit are about 30 cm in length and 20 cm in diameter. Weight varies but usually is between 1 and 3 kg. The breadfruit tree is resistant to wind damage and disease, may live for 75 years or longer and no major cultivation care is required. One tree can produce 300 to 500 fruits/year in two crops. Normally the fruit is peeled and the heart and stem are removed and, in most cases, discarded. This resulted in an overall yield of edible pulp of about 68% of the total fruit weight, although this varies with stage of maturity (Graham and Negron, 1981).

Normally, the mature fruit is preferred for human consumption. A mature fruit can be differentiated by appearance (Thompson et al., 1974). Mature fruit has dark green, large polygonal segments, as compared to smaller, densely packed segments for immature fruits. A very mature fruit softens and can be detected manually. A very immature fruit exudes a large amount of latex when peeled and has an undesirable taste. Processing alternatives such as breadfruit flour (Arceley and Graham, 1984), fermentation (Cox, 1980) and freezing (Passam et al., 1981) have been investigated. In view of the ready availability of breadfruit, its chemical and compositional similarity to potato, utilization forms similar to other starchy crops seemed a reasonable possibility. A chip-type snack similar to potato, plantain, corn or cassava chips was the focus of our research.

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MATERIALS & METHODS

DUE to their unavailability in South Florida and their perishable nature, breadfruits were shipped by air from Puerto Rico. Shipments of both yellow and white heart types (specific cultivar unknown) were used. Figure 1 outlines the study. Upon receipt fruits were inspected; over- or under-ripe fruits discarded, and blemished portions removed. The solid flesh was passed through a food slicer set to cut even slices. Preliminary experiments established optimum slicing thickness as 1.25 mm and partially hydrogenated soybean oil frying temperature of 165°C for 30–40 sec for each side. Drying trials to reduce oil pick-up consisted of placing slices in an air dryer at 57°C up to 40 min. Fried breadfruit chips were blotted on absorbent paper towels and salted, while still warm, with Alberger fine flake salt (Diamond Crystal Salt Co.) at 1.0 to 2.0% by weight.

The chips were then packed into metallized, commercial film 75 gauge oriented polypropylene/polyethylene/70 gauge aluminum, coextruded 70 gauge polypropylene (Bryce Corp., Memphis, TN) and hermetically sealed in air without vacuum or nitrogen flush. (These conditions were used because, in high producing breadfruit regions of the world (developing countries), production methods would most likely be less sophisticated than those practiced for potato chip production in highly developed industrialized nations). Packaged samples along with commercials packs of potato and plantain-chips were stored at 2, 27 and 55°C. At 3-day intervals samples of all three types were removed for analysis.

Analyses conducted on freshly prepared or purchased chips and on selected stored samples were: moisture by the toluene distillation method (Gould, 1986) oil content by soxhlet extraction (Lulai and Orr, 1979), peroxide value and free fatty acids by AOCS methods (Min and Schweitzer, 1983), and salt by Mohr titration (Gould, 1986).

Freshly prepared breadfruit chips (< 1 wk old) and commercial samples of potato and plantain chips were evaluated by a 20-member sensory panel employing a 9-point hedonic scale for color, texture, flavor and overall acceptability. A similar panel evaluated the same products at the Univ. of Puerto Rico. Panelists at both locations were unfamiliar with breadfruit chips. Few of the UF but all of the UPR judges were familiar with breadfruit. Most panelists participated routinely in sensory evaluations, but were not preselected nor trained for this study.

Data were subjected to analysis of variance using a SAS program.

RESULTS & DISCUSSION

THE DELICATE NATURE and extreme perishability of breadfruit were evident from the condition of the samples received by air freight. Although fruits were carefully selected and packed for shipment and handled rapidly upon receipt, up to 50% were unusable, being either slightly immature or overmature—too soft for chipping. In addition, blemished por-

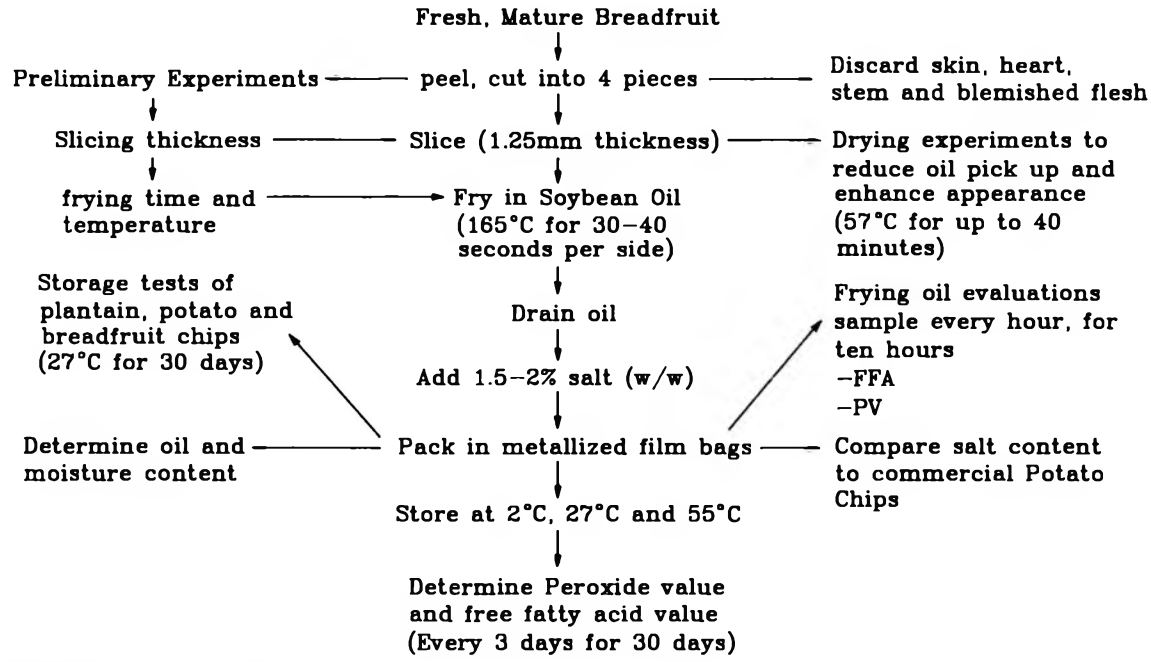

Fig. 1—Breadfruit process and analysis flow scheme.

Table 1—Effect of drying prior to frying on oil content of breadfruit chips

Drying time (min)	Oil $(\%)_a$
0	42[a]
10	36[a]
20	31[b]
30	26[b]
40	20[c]

[a-c] Means in column followed by same letter not significantly different at $P \leq 0.05$.
Total observations = 10

tions, indicative of physical damage, had to be removed and delays in delivery of over 24 hr often resulted in unacceptably soft fruit. Other utilization options exist for soft flesh (e.g. extruded or dough-based items), but they were not explored.

Since no information was available on breadfruit chip technology, studies on potato chips and other snacks served for reference (Talburt and Smith, 1975; Gould, 1986; Gamble and Rice, 1987; Lawson, 1985; Min and Schweitzer, 1983). Fruits averaged about 2 kg fresh weight each and after slice preparation and frying a pulp yield was about 67% (fresh weight basis). A slice thickness of 1.25 mm was dictated by the brittle nature of thinner chips and uneven texture and moistness of thicker slices. The 165°C frying temperature for 30–40 sec on each side consistently produced a light colored, crisp chip. The chips had about 3% moisture and oil content of 42%. The oil level was slightly higher than that observed in commercial potato and plantain chips (~38%).

In an attempt to reduce oil level, breadfruit chips were predried prior to frying. A dramatic decline in oil absorption was observed (Table 1). However, unacceptable darkening and a scorched flavor resulted after 30 min of predrying at 57°C. Therefore, 20–30 min predrying is recommended (for oil reduction). Despite this oil reduction technique and manipulations of the frying time-temperature regime, many chips still had an oily, translucent appearance in the central region. This appearance did not adversely affect texture or flavor, although it could detract from visual appeal.

The salt content of breadfruit chips was about 1.5% which was comparable to that of four brands of commercial potato chips where salt content ranged from 1.3–1.6%. Informal comments from tasters suggested that a 1.5–1.75% salt level was preferred, (although, for lowering dietary sodium reduced salt

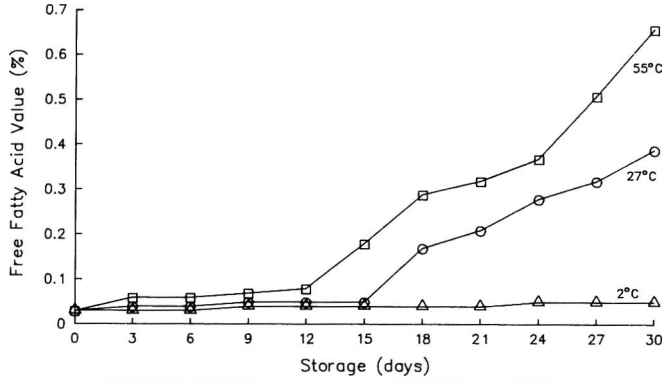

Fig. 2—Free fatty acid values of breadfruit chips: effects of storage temperature and time.

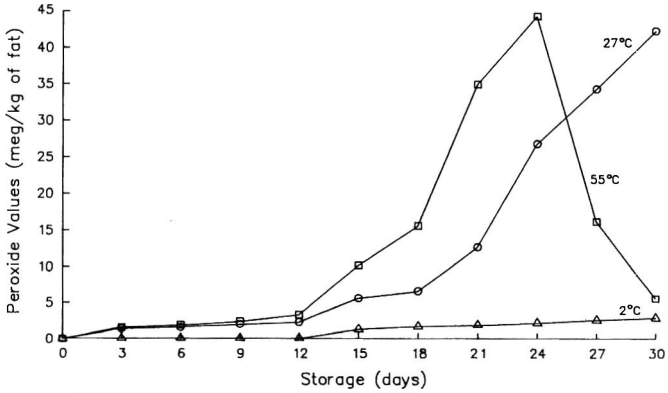

Fig. 3—Peroxide values of breadfruit chips: effects of storage temperature and time.

level could be used but may require additional acceptability studies).

The lipid oxidative stability of breadfruit chips was clearly influenced by storage temperature as reflected by free fatty acid (Fig. 2) and peroxide (Fig. 3) values. Rancidity was detected in the chips after 21 days at 27°C when the peroxide

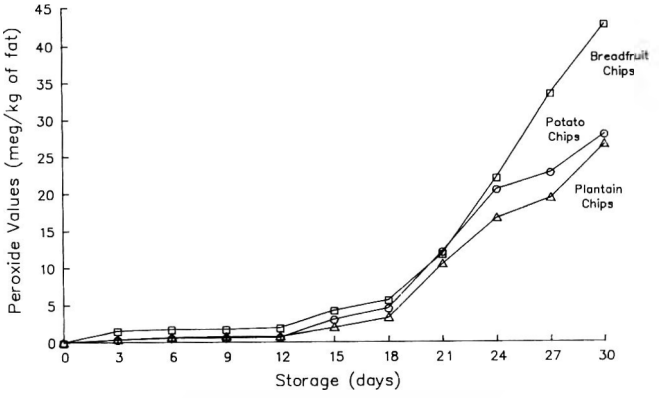

Fig. 4—Peroxide values of breadfruit chips: effects of storage temperature and time.

Table 2—Sensory evaluation of breadfruit, plantain, and potato chips

	Color[c]		Flavor[c]		Texture[c]		Overall acceptability[c]	
Chip	UF	UPR	UF	UPR	UF	UPR	UF	UPR
Plantain	6.3[a]	6.0[a]	7.0[b]	7.7[a]	7.0[a]	7.5[a]	6.9[a]	7.3[a]
Potato	7.8[b]	7.7[b]	7.8[a]	8.2[a]	7.5[a]	7.7[a]	7.0[a]	7.0[a]
B'fruit	7.5[b]	7.5[b]	6.8[b]	7.0[b]	7.4[a]	7.4[a]	6.6[a]	7.0[a]

[a,b] Means in columns followed by same letter not significantly different at $P \leq 0.05$. Total observations = 40

[c] Higher value indicates more desirable ranking for each attribute.

value reached 12. Figure 4 illustrates lipid deterioration as compared to potato and plantain chips. Rates were similar for about 24 days, after which rancidity in breadfruit chips accelerated. The peroxide value decline after 24 days at 55°C presumably reflected peroxide breakdown at that extreme temperature (Hamilton and Rosell, 1986). Since the chips were prepared under noncommercial conditions and packed in air, our storage study represented sub-optimal circumstances, somewhat representative of developing countries with abundant breadfruit. Use of a nitrogen gas flush and judicious application of antioxidants should extend shelf life notably (Asap and Augustin, 1986; Noor and Augustin, 1984). The frying oil, after being subjected to 10 frying cycles of 1 hr each, reached a 0.32% free fatty acid level and a peroxide value of 7. This slow deterioration was reduced substantially by filtering out chip particles between frying runs.

Sensory evaluations of the three chip products are summarized in Table 2. Panelists at the Universities of Florida and of Puerto Rico ranked breadfruit chips slightly but not significantly lower in overall acceptability than the commercial products. The acceptability scores of "like slightly" to "moderately" were encouraging. There appeared to be no inherent quality defects in breadfruit chips and they were well received in both formal and informal tastings.

CONCLUSION

BREADFRUIT is well suited as a snack ingredient. Provided attention is given to maturity selection, predrying, frying, salting, packaging and storage, breadfruit chips could be an effective value-adding alternative for this underutilized food resource.

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Aciduric, pH-Elevating *Bacillus* which Cause Noneffervescent Spoilage of Underprocessed Tomatoes

JAMEEL AL DUJAILI and ROBERT E. ANDERSON

ABSTRACT

In the preliminary phase of this study, a selective isolation medium was developed. Using the new medium, 24 strains of *Bacillus* Spp. that elevate tomato juice pH were isolated from garden soil or natural vegetative materials. Two jars of home-canned, water-bathed tomatoes contained sporeformers which produced noneffervescent spoilage. All strains were morphologically and physiologically different from 2 flat-sour strains of *B. coagulans* and *B. licheniformis* strains, but similar to a previous isolate from home-canned, spoiled green beans that elevated pH. The new medium may have future applications in isolation and enumeration of other acidurics including yeast-like microorganisms.

Key Words: bacillus, aciduric, effervescence, sporeformers, bacteriology-media, tomatoes

INTRODUCTION

THE LOW pH of tomatoes and their products (4.2 – 4.5) has been a vital help in heat processing to prevent spoilage and consequent outgrowth of certain foodborne pathogens (Sapers et al. 1977; Huhtanen et al. 1976). Some spoilage bacteria however, increase pH, thus possibly causing botulinal hazards. Montville (1982) showed that contamination by *Bacillus licheniformis* resulted in elevation of tomato juice pH above 4.6, and thus stimulated outgrowth of associated *Clostridium botulinum* spores and toxin production. Fields et al. (1977) obtained an isolate from spoiled, underprocessed home-canned green beans that subsequently increased pH of a tomato serum broth. The isolate was presumptively identified as *B. coagulans* 064-T-08, but no inference was drawn to its potential as a botulinal hazard in tomato products. Anderson (1984) found the Fields' strain (FS) not only grew well anaerobically in heat-processed tomato juice, but elevated juice pH from 4.40 to 5.05 during 6 days incubation at 35°C.

The etiology of pH-elevating strains of sporeformers, possibly intermediates between classical *B. coagulans* and *B. licheniformis* remains poorly understood. To our knowledge, no research has been reported to compare and evaluate common properties of strains similar to the FS. The objectives of our research were to conduct a preliminary survey to determine natural occurrence of sporebearers similar to the FS using a newly developed selective isolation medium. We also compared their morphological and physiological characteristics with those of related strains of known *Bacillus* species.

MATERIALS & METHODS

Sampling

For isolations from plant leaf surfaces, moistened sterile swabs were individually rubbed over 10 × 10 mm leaf surface areas and then streaked onto petri plates (15 × 150 mm) of prepared isolation medium. All vegetation was selected from common accessible garden areas unless otherwise mentioned (Table 1). Soil was sampled at random from on-campus floral beds, home gardens and the university farms. At each site approximately 3 kg of soil were collected and well mixed. Twenty grams of each sample were carefully mixed in the laboratory on the day of collection. A lg subsample was transferred to 10 mL sterile 0.1% peptone water diluent. From this mixture, 0.1 mL was transferred to each of 3 plates of selective isolation medium and streaked over the plate. This plating procedure was repeated in triplicate for each soil sample.

Nearly spherical potatoes and tomatoes of about baseball size (ca 23 cm diam) were selected at random from freshly harvested produce from the university farms. Two potatoes and 2 paired tomatoes served as individual subsets of each. These were individually swabbed with sterile cotton swabs (moistened previously with sterile 0.1% peptone water). The 2 swabs from the potatoes of each subset or those from each of the tomatoes were separately streaked on prepared isolation medium.

Isolation medium

Because the desired isolates would be aciduric or acidophilic, a selective medium, Bacillus Tomato Juice Agar (BTJA), was developed for isolation (Table 2). As an adjunct, we chose commercial tomato juice which, in our experience, gave faster growth of reference cultures than basal medium acidified with either citric or hydrochloric acids. Sorbic acid was evaluated at various dilutions and effectively

Table 1—Origin and isolation frequency of pH-elevating Bacillus *strains from various natural sources*

Source	Frequency	Designated strain no.
Soil	4/20	2-86 5-87 25-87 29-87
Whole canned tomatoes[a] (Home canning)	2/2	1-86 4-86
Potatoes[b]	7/35	PO_1-87 PO_2-87 PO_3-88 PO_4-88 PO_5-88 PO_6-88 PO_7-88
Tomatoes[b]	5/11	T_1-88 T_2-88 T_3-88 T_4-88 T_5-88
Unwashed lettuce[c]	1/4	L-88
Dried Pepper[c] (powder)	1/4	3-86
Green bean leaves[d]	1/3	10-86
Squash leaves[d]	1/4	11-86
Weed leaves[e]	2/23	24-86 30-86
Total	24/105	

[a]Morgantown vicinity, from a homemaker's kitchen
[b]West Virginia University Farms
[c]Retail market, Morgantown
[d]Home garden, Robert Anderson
[e]Flower bed, Pensacola, Florida

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Table 2 – Composition of bacillus tomato juice agar (BTJA)[a]

Phytone TM peptone	10.0
Brom cresol purple	0.02
NaCl	5.0
Agar	30.0
Potassium sorbate	0.15
Tomato juice	400 mL
Distilled water	600 mL

[a] Values are in g/L, except for tomato juice and distilled water. pH was adjusted to 4.35 before autoclaving.

employed as an inhibitor for yeasts and molds. Bromcresol purple was incorporated to indicate pH elevation during growth. The newly developed BTJA was used extensively throughout the isolation phase.

Criteria for isolation

Desired colonies were those that grew on BTJA and produced zones of purple in the yellow, acidified medium. Single colonies with a purple margin and background were carefully picked for restreaking on BTJA if they had a dry, flake-like appearance similar to the reference FS. All isolates were examined by Gram and spore stains as were control cultures.

Control cultures

B. coagulans 064-T-08, *B. licheniformis* B 110, and *B. coagulans* NRS 54 were used as reference cultures. All cultures were aerobically grown on trypticase soy agar slants (TSA) (BBL Microbiology Systems, Cockeysville, MD) or BTJA at 44°C. Stock cultures were maintained at 7°C in 16 × 125 mm screw cap tubes on TSA slants.

Morphology

One to 2 day old subcultures grown on TSA were examined for colonial morphology. Cell morphology of isolates was determined with light and also by phase contrast microscopy (Nikon Optiphon, Tokyo, Japan). For the latter, cells were added to a precoated agar slide. After placement of a cover glass, cells were observed under oil immersion-phase contrast conditions.

Physiology

All isolates were tested for growth in litmus milk and glucose broth. Five of the isolates, 1-86, 2-86, 3-86, PO_1-87, and PO_2-87, were arbitrarily selected for pH and temperature ranges for growth. All inocula were from 1–2 day old cultures on TSA slants. All pH determinations during growth were made with a Fisher model SO-B-107 pH meter (Fisher Scientific, Pittsburgh, PA). It was standardized prior to use each day with Fisher certified buffers at pH 4.00 and 7.00. Temperature ranges for growth were determined using nutrient agar slants (Difco Laboratories, Detroit, MI) in 16 × 150 screw cap tubes. These were separately kept in an air incubator at 10, 20, 25, 35, 40, 45, 50, 55, 60 or 65° C and observed daily for onset and comparative, sustained growth for 21 days (before declaring no growth at a specific temperature). Sodium chloride tolerance was determined with nutrient broth containing separate levels of 2, 5, 7 and 10% (W/V) NaCl, incubated in duplicate sets at 30 and 44°C for 14 days (before declaring any tubes negative for growth). A modified citrate agar was employed, with citrate utilization indicated by an alkaline shift of the phenol red indicator during 15 days incubation. Inoculated litmus milk was incubated at 44°C for 1 and 2 days to observe reduction and proteolysis (characteristic of strains similar to the FS reference culture) and other changes in the medium. All isolates were tested for catalase using 3% H_2O_2. These strains were also screened for lysine decarboxylase (Moeller, 1955).

Glucose fermentation was determined from growth in a basal broth containing, per liter of distilled water: 1.0g $(NH)_2$ HPO_4, 0.7g yeast extract, 0.2g KCl, 0.2g $MgSO_4$. $7H_2O$ and 15 mL of a 0.04% W/V solution of bromcresol purple. After autoclaving the basal broth, filter-sterilized glucose was aseptically added for a final concentration of 1% (W/V). Isolates were then individually inoculated and subsequently observed for growth and acid production after 1, 2, 3 and 4 days incubation at 44° C. Inverted Durham tubes were used to detect gas production.

RESULTS & DISCUSSION

TWENTY-FOUR ISOLATES similar to the FS were obtained from natural sources: 7 strains from 35 subsets of raw potatoes; 5 from 11 subsets of raw tomatoes; and 2 from home-canned tomatoes. The latter were water-bathed, cooked in quart canning jars. Altogether, isolates were obtained from each of the 9 sites tested (Table 1). The 24 isolates showed remarkable resemblances. All produced flake-like pellicles in TSB cultures after 2 days incubation at 44°C. Morphologically and physiologically, they were quite similar to FS, yet considerably different from any of the flat-sour strains of *B. coagulans* or the cultures of *B. licheniformis* in our possession. All isolates produced powder-dry, flake-like colonies with irregular margins within 24 hr. Whereas, 24 hr colonies of *B. licheniformis*, B 110 were white and gummy or mucoid in texture. Flat-sour colonies of *B. coagulans* NRB 54 appeared moist, flat and had round, regular margins (Table 3).

All of the isolates were lysine decarboxylase positive and reduced litmus milk. Other physiological similarities to FS are shown in Table 4. None of the 24 strains grew at 10 or 60°C; all had optima at or near 45°C. While none grew at pH 4.2, all had optima pH range of 5.00 to 5.70 in BTJB. Strains PO_1-87 and PO_2-87 were negative for citrate utilization as was the FS; other isolates however, utilized citrate as sole carbon source. Anderson (1984) had found FS grew well in tomato juice and later Shamsudin (1984) prescribed purple broth base (BBL Microbiology Systems, Cockeysville, MD) as a basal growth medium. These observations were applied in development of the isolation medium, BTJA.

In BTJA the tomato juice provided acidity for selectivity and additional nutrients for growth of aciduric sporebearers. Other adjunct acids, *e.g.* citric and hydrochloric, did not support initial good growth of the FS or our reference cultures of *Bacillus*. Sorbate was necessary to control yeasts and fungi, and the effective level (Table 2) was determined by trial and

Table 3 – Morphology of Bacillus *isolates*

Characteristics	*B. coagulans* NRS 54	Isolates	*B. licheniformis* B110
Gram's stain	+	+	+
Rods			
width, um	0.6 – 1	0.6 – 0.95	0.6 – 0.8
length, um	2.5 – 5	2.5 – 5	1.5 – 3.0
Colonies	Grayish-white, moist, flat and translucent margins round.	Dirty-cream colored, powder dry, flake-like appearance, margins irregular, wavy.	Dull, white, opaque, attached strongly to agar surfaces, margins irregular, mucoid.
Sporangium swollen	+	+	–
Spore shape[a]	E (R/O)	E (R/O)	E (R/O)
Spore position[a]	C/T	C/T	C

[a] (E) Ellipsoidal; (R/O) Round-oval: (C/T) Central terminal; (C) Central.

Table 4—Physiological characteristics of Bacillus *isolates with comparisons to reference cultures after 7 days at 44°C*

Characteristics	*B. coagulans* (NRB 54)	*B. licheniformis* (B110)	Fields' strain (064-T-08) (FS)	All isolates
[a] Growth in 7% NaCl broth	–	+	–	–
Catalase	+	+	+	+
Acid, no gas from glucose	+	+	+	+
[b] Litmus milk reaction	A,P	R,P	R,P	R,P
Lysine decarboxylase	–	–	+	+

[a] All negative tubes were incubated for 21 days.
[b] A = acid; R = reduction; P = slight proteolysis.

error. Nine various concentrations of sorbic acid, 0.100 to 0.005% were evaluated using 3 mold cultures in our possession before deciding on 0.15g/L (Table 2). Actodione was tried as a fungistat and found ineffective. The indicator, bromcresol purple provided satisfactory color change to identify colonies that elevate pH by some other mechanism.

All of our isolates were strongly lysine decarboxylase positive, grew in 5% NaCl broth, but not in 7% and did not produce gas during glucose fermentation. In contrast, *B. licheniformis* B-110 did not possess lysine decarboxylase and grew in 7% NaCl broth. Similar to our strains, the flat sour strain of *B. coagulans* NRB 54 did not grow in 7% NaCl broth, but *unlike* ours, was negative for lysine decarboxylase. From this preliminary survey results show a uniform distribution in nature of pH-elevating sporebearers similar to FS. Strains were readily isolated from surfaces of soil-contact vegetables, from plant leaves and in garden soil. Thus, such strains were apparently rather commonplace in nature.

Many reports describe the incidence, coexistence, and opportunistic associated growth of aciduric bacteria and fungi in and on tomatoes (Berry, 1933; Thompson, 1981, Gordon et al., 1973; Williams and Maki, 1980, Montville, 1982). If tomatoes (or other garden produce) are not washed beforehand, spores will be added during filling of jars prior to canning. These spores, if from nongas-producers, could possibly survive and cause initial non-effervescent spoilage. Two of our isolates came from spoiled, noneffervescent home-canned tomatoes. The ambient juice pH of one of those samples was 5.1 and the other, 4.8. Thus, the contents of those jars posed a possible bacteriological hazard with respect to germination and growth of *Cl. botulinum* spores.

There have been several other reports of sporebearers surviving inadequate heat processing of tomatoes (Becker and Pederson, 1950; Denny, 1981; Williams and Maki, 1980; Berry, 1933; Mundt et al. 1978). Thus, it is important that USDA recommendations for home canning be closely followed. Recently, one of us (Anderson, unpublished data, 1990) received 2 quarts of spoiled effervescent home-canned beans from a batch of 190 quarts. A homemaker mistakenly processed them at 9 lb pressure instead of the prescribed 12 lb for 25 minutes. (She had not obtained current guidelines on canning of beans.) The two jars we received were negative for *Bacillus*, and there was no proof that strains similar to ours were involved in the spoilage. However, the incident served to illustrate the hazard or risk of arbitrary canning at home.

In the case described, fortunately opportunistic heterofermentive spoilage bacteria typically produced visible gas and thus were indicators of underprocessing. However, our 2 isolates from home-canned tomatoes produced no visible gas, and further, there was no effervescence in the jars to indicate spoilage. Cloudiness was the only sign of abnormality along with elevated pH. Hence, spoilage would be less obvious to the untrained consumer eye. Just as the FS was previously isolated from underprocessed home-canned beans (Fields et al. 1977), similar pH-elevating contaminants represent a considerable risk of noneffervescent spoilage of borderline, under-processed tomatoes and beans.

The isolates of this research appeared both morphologically and physiologically different from either *B. coagulans* or *B. licheniformis*. Studies of plasmid profiles (unpublished data 1990) showed other differences between those reference species and our isolates. Further work is needed before one could conclude pH-elevating strains similar to the FS belong in a separate subgroup or subspecies. However, they, like the FS, were not flat sour in nature and therefore, not typical strains of *B. coagulans*. Further work is underway to identify the mode of pH elevation and additional distinguishing features of our isolates.

CONCLUSIONS

ISOLATES of nongas-producing sporeformers (24) were obtained from 105 garden samples with the greatest frequency, 5/11 from tomatoes. The major consumer safety concern of this subgroup of aciduric noneffervescent sporebearers was their ability, in absence of visible gas, to latently elevate pH to levels that may facilitate germination of ubiquitous *Clostridium* spores. A new differential selective medium, BTJA, may have applications for isolation and enumeration of other aciduric microflora, especially acid oxidizers and perhaps lactics. All 24 isolates resembled the FS, a surviving isolate from home-canned green beans but all, including the FS, differed markedly from the reference cultures *B. coagulans* and *B. licheniformis*.

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Juice Extraction Process and Apple Cultivar Influences on Juice Properties

M. CLIFF, M. C. DEVER, and R. GAYTON

ABSTRACT

Effects of several processing methods (oxidative, nonoxidative, diffusion extraction, enzyme liquefaction) on varietal apple juice character was examined. Analytical evaluations included titratable acidity, soluble solids, pH, phenol, suspended solids, turbidity, Hunterlab color, organic acids and sugars and showed definite extraction process and cultivar differences. Sensory evaluation identified eight attributes, color, clarity, fruity aroma, oxidized, fruity flavor, sweet, sour, astringent, as significant factors in characterizing differences among the juices. Canonical discriminant analyses (CDA) of analytical data showed correct allocation of all juices to both process type and variety. CDA of sensory data, revealed a significant separation of processes but not all cultivars.

INTRODUCTION

APPLE JUICE EXTRACTION in North America has traditionally been a mechanical process whereby apples are milled and pressed. Under optimum conditions juice yield is 70–80% (w/w) but may be below 65% (w/w) for stored apples. Due to rising costs of raw material and production there has been a need for a higher yielding, more efficient juice extraction processes. In response to this, two new physical processes, diffusion extraction and enzyme liquefaction, were developed.

Extraction process affects both the analytical and sensory characteristics of the juice. For example, Wucherpfenning (1981) found lower titratable acidity and higher ash, mineral, and phenol concentrations in juices produced by diffusion extraction of German cider apples compared to juices extracted by pressing. Although aromas of the two juices did not differ, 'diffuser juice' was sour and astringent. Binkley and Wiley (1978) showed 'pressed juices' were preferred over 'diffuser juices' and concluded that the diffusion process had a negative effect on juice quality. Dörreich (1983) found that juice produced by enzyme liquefaction had increased soluble solids and mineral content. Those juices had a characteristic apple flavor; however, those incubated at higher temperatures (45–50°C) before extraction had a weak flat aroma.

Research has compared juices extracted by the new physical processes, (diffusion extraction or enzyme liquefaction) with those extracted by pressing and, it is well known that both process and cultivar affect juice character. However, the relative influence of extraction process vs cultivar on juice properties has not been documented nor have four different juice extraction processes been directly compared. Our objective was to examine the effects of both cultivar and process type on juice properties using analytical and sensory techniques.

MATERIALS & METHODS

Juice extraction

One bin (250 kg) of each of four apple cultivars (McIntosh, Golden Delicious, Delicious, and Spartan) was obtained from Agriculture Canada, Summerland Research Station orchards. Apples were harvested at commercial maturity and stored in air (1°C, 70–80 RH) until processing (2 mo). From each cultivar, juice was extracted using 4 processes including; (1) an 'oxidative' (without inhibition of polyphenol oxidase) mechanical extraction, (2) a 'nonoxidative' (with inhibition of polyphenol oxidase) mechanical extraction, (3) an enzyme liquefaction extraction, and (4) a diffusion extraction.

Nonoxidative pressing. Apples were milled, (hammermill; screen hole size 1.25 cm.) treated with 1000 mg/kg ascorbic acid, and pressed using a rack and cloth hydraulic press.

Diffusion extraction. Apples were sliced using a Hobart slicer fitted with corrugated slicer blades. Apple slices (2.5 mm) were fed through a De Danske Sukkerfabrikker (DDS) pilot diffuser (Type B) (De Danske Sukkerfabrikker, Copenhagen, Denmark) at 30 kg/hr. The countercurrent water flow was operated at 24 kg/hr. Operating temperature was 70°C. Samples were withdrawn when sugar levels at the outlet had stabilized. Diluted juice was later concentrated back to single strength (based on soluble solids determined on original sample) by freeze concentration.

Enzyme liquefaction. Apples were milled and pumped through a Cherry-Burrell scraped surface tubular heat exchanger and heated to 42–45°C. The mash was mixed with 0.02% Pectinex Ultra SP (Novo Industri A/S, Bagsvard, Denmark), incubated at 45°C for 2.5 hr and then centrifuged using an Alfa Laval decanter centrifuge (Model NX 309S-31G Scarborough, Ont., Canada).

Oxidative pressing. Apples were milled and pressed using a Vetter screw press (Type 1/2 Vetter Maschinefabrik Gmbh, Kassel-Dettenhasen, Germany). Liquid Irgazyme (0.02% Novo Industri A/S) was added to the juice which was held at 0.5°C for 12 hr and then racked to remove precipitated solids. Juices from all except the diffuser process, were flash 'pasteurized' (30 sec, 90°C) using a coil heat exchanger (Beveridge and Harrison, 1986) then frozen (−18°C) for further analysis.

Juice analysis

The pH of the juice was determined with a Radiometer pH meter (Radiometer, Cooenhagen, Denmark) and the titratable acidity (TA) by automatically titrating a 2 mL sample with 0.1N NaOH to pH 8.2. TA was expressed as g malic acid/L. Soluble solids were determined in °Brix using an Abbe refractometer (Reichart Scientific Instruments, Buffalo, NY) and suspended solids were calculated on a percent wet weight basis after centrifuging 50g samples at 3,000×g for 10 min. Total phenols were evaluated spectrophotometrically (Varian DMS 100 UV Visable Spectrophotometer, Varian Techtron Pty. Limited, Mulgrave, Victoria, Australia) by the Folin-Ciocalteu method (Amerine and Ough, 1980), and expressed as gallic acid equivalents (GAE)/100 mL. Turbidity (absorbance at 600 nm) was determined after centrifuging the samples to remove coarse solids (1000×g, 3 min). A Hunterlab Model D25 Optical Sensor (Hunter Associates Laboratory Inc., Reston, VA) was used to measure juice color on a 50 mL sample placed in a 10 cm ring mounted on a Hunterlab standard white plate. The instrument was standardized with Hunterlab white standard No. C2-18593 and the light source was a DZA low voltage halogen cycle lamp.

Sugars (sucrose, glucose, fructose) and organic acids (sorbic, citric, galacturonic, malic, quinic) were analyzed with a Waters 490 HPLC system (Waters HPLC Millipore (Canada) Ltd., Mississauga, Ont., Canada) using methodology adapted from Beveridge et al. (1986). Juice samples were centrifuged (12,000×g, 10 min) filtered (0.45 μm) and passed through a Bio-Rad AGIX8 (acetate) resin bed (Bio-Rad Laboratories Canada Ltd., Mississauga, Ont.) Sugars were eluted from the resin with water into a 25 mL volumetric flask containing mannitol as internal standard. The sugar separation used a Brownlee Polypore anion guard column (Brownlee Labs Inc., Santa Clara, CA) and Polypore CA 10 μm ion exchange column with water as mobile phase (65°C, 0.3mL/min). Organic acids were eluted from the resin column with 1.5N sulphuric acid into a 10 mL volumetric flask containing fumaric acid as internal standard. The analysis used two Bio-

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Table 1—Apple juice references for anchoring linescales.

Sensory term	Reference Cultivar	Process
Visual		
color (yellow)		
light	McIntosh	nonoxidative
medium	Golden Del.	enzyme liquefaction
dark yellow (brown)	Del.	oxidative
clarity		
cloudy	Spartan	nonoxidative
transulent	Golden Del.	diffusion extraction
clear	Spartan	oxidative
Aroma		
fruity aroma	Del.	oxidative
oxidized/phenolic	Golden Del.	enzyme liquefaction
Flavor		
fruity flavor	Golden Del.	oxidative
sweet	McIntosh	nonoxidative
sour	Golden Del.	oxidative
astringent	Spartan	enzyme liquefaction

Rad columns (Aminex HPX-87H) fitted in series with a Brownlee cation guard column and 0.01N sulphuric acid (65°C, 0.6 mL/min) as the mobile phase. Both sugars and acids were detected using a differential RI detector.

Sensory evaluation methods

Using descriptive analysis with 10 cm linescales, 16 juices (4 processes × 4 cultivars) were profiled for visual, aromatic and flavor characteristics. These were identified in an initial screening and internal references were selected (Table 1). The judges, twelve Agriculture Canada staff members, were familiarized with the juices and were asked to verify the appropriateness of the sensory terms and references. In each of two training sessions, they practiced 'flavor' recall by matching coded samples to internal references. Judges were given immediate feedback on their performance and further practice if necessary.

Test samples were evaluated in triplicate using a randomized complete block design, at 6 samples/day for 8 days. Samples (30 mL) were served at room temperature (23°C) in covered tulip shaped glasses and were evaluated in isolated booths containing a complete set of references. Visual characteristics were scored in clear glasses under natural light whereas aroma and flavor terms were scored in black glasses under incandescent light. Judges evaluated each sample across all terms before proceeding to the next. The linescales for color and clarity were anchored at the mid-point and either end (light/dark and cloudy/clear respectively). Aroma and flavor linescales were anchored at 9 cm with the internal reference. Judges rinsed between samples and all samples were expectorated.

Data analysis and statistical methods

Analysis of variance (ANOVA) Statistical Analysis System (SAS 1985) was used to determine analytical and sensory differences among juices. A preliminary 3-way (judge, process, rep) ANOVA of sensory data showed replication to be nonsignificant and so replication variance was pooled into the error and a 2-way (judge, process) ANOVA calculated. For several of the terms, poor judge consistency resulted in a significant judge × process interaction. When the F-ratio was recalculated according to the technique of Goniak and Noble (1987), process differences were still significant. Canonical discriminant analysis (SAS Proc Candisc SAS, 1985) of the significant analytical terms ($p < 0.05$) and mean sensory data used process type and apple cultivar for class differentiation. This analysis determines linear combinations of variables that summarize the differences among the classes.

RESULTS & DISCUSSION

JUICE EXTRACTION PROCESS and apple cultivar were shown to affect juice character. Many analytical process differences (Table 2) were consistent for all varieties. For ex-

Table 2—Mean analytical values for apple cultivars (n = 2)

		Golden delicious						Red Delicious					
		Non-oxidative	Enzyme liquefaction	Diffusion extraction	Oxidative	LSD	Level of sig	Non-oxidative	Enzyme liquefaction	Diffusion extraction	Oxidative	LSD	Level of sig
TA	mg malic/100mL	0.50	0.58	0.38	0.50	0.10	***	0.23	0.37	0.19	0.25	0.01	***
soluble solids	°Brix	14.1	13.7	11.2	12.0	0.6	***	13.2	14.4	13.7	13.5	0.3	***
pH		3.50	3.85	3.64	3.49	0.05	***	3.93	3.61	4.13	3.97	0.02	***
suspended solids	%(w/w)	2.2	0.9	1.2	0.4d	0.2	***	0.5	0.6	1.3	0.5	0.0	NS
total phenols	mg GAE/100mL	726	485	658	282	92	**	950	740	916	427	205	**
turbidity	A^{600}	2.10	0.56	0.72	0.06	0.04	***	0.34	0.19	0.49	0.19	0.07	***
Hunterlab L		64.5	56.2	60.5	61.8	1.4	***	65.3	55.3	39.6	60.0	6.0	***
Hunterlab a		−4.4	0.2	−1.2	−3.8	1.0	***	−2.3	4.0	13.6	−0.8	2.0	***
Hunterlab b		31.2	30.2	19.6	24.2	0.4	***	3.1	26.1	23.4	30.2	7.4	**
sucrose	g/100mL	3.90	2.91	2.73	2.89	0.11	***	2.74	2.33	3.10	2.60	0.12	***
glucose	g/100mL	1.51	1.89	1.44	1.40	0.10	**	2.75	2.50	1.68	2.14	0.27	***
fructose	g/100mL	6.92	6.80	5.68	5.92	0.29	***	7.09	6.18	6.92	5.97	0.36	**
sorbic acid	g/100mL	0.19	0.16	0.15	0.13	0.04	**	0.18	0.17	0.19	0.15	0.07	NS
galacturonic acid	g/100mL	0.02	0.24	0.00	0.00	0.00	***	0.00	0.22	0.00	0.00	0.01	***
malic acid	g/100mL	0.59	0.54	0.49	0.53	0.02	**	0.25	0.34	0.25	0.37	0.04	**
		McIntosh						Spartan					
		Non-oxidative	Enzyme liquefaction	Diffusion extraction	Oxidative	LSD	Level of sig	Non-oxidative	Enzyme liquefaction	Diffusion extraction	Oxidative	LSD	Level of sig
TA	mg malic/100mL	0.52	0.67	0.42	0.60	0.03	***	0.47	0.58	0.21	0.45	0.01	***
soluble solids	°Brix	10.8	12.3	9.05	11.4	0.4	***	14.0	14.2	13.0	12.7	0.17	***
pH		3.38	3.26	3.52	3.34	0.04	***	3.65	3.45	4.08	3.61	0.05	***
suspended solids	%(w/w)	1.06	0.8	1.4	0.5	0.1	***	1.6	0.8	1.1	0.6	0.3	**
total phenols	mg GAE/100mL	975	489	747	308	191	**	766	386	783	300	74	***
turbidity	A^{600}	1.12	0.33	0.59	0.06	0.06	***	1.66	0.43	0.54	0.14	0.02	***
Hunterlab L		65.6	56.5	53.2	63.3	0.42	***	65.5	53.5	43.6	60.7	0.6	***
Hunterlab a		−0.6	2.3	6.9	−2.3	1.4	***	−2.5	4.2	11.6	−2.5	0.5	***
Hunterlab b		10.5	25.9	23.0	23.2	0.73	***	19.2	28.9	26.4	25.7	0.7	***
sucrose	g/100mL	2.13	2.24	1.71	2.31	0.22	**	3.45	2.93	2.54	3.33	0.29	**
glucose	g/100mL	1.17	1.33	1.14	1.08	0.13	*	1.71	1.72	2.18	1.39	0.21	**
fructose	g/100mL	6.52	6.71	5.72	5.93	0.44	**	7.31	6.99	6.60	6.99	0.66	*
sorbic acid	g/100mL	0.11	0.15	0.11	0.12	0.02	*	0.21	0.18	0.16	0.16	0.02	**
galacturonic acid	g/100mL	0.00	0.28	0.00	0.00	0.02	***	0.00	0.25	0.00	0.00	0.01	***
malic acid	g/100mL	0.63	0.76	0.54	0.55	0.12	*	0.54	0.52	0.37	0.50	0.04	***

* Significant at 0.05; ** Significant at 0.01; *** Significant at 0.001.

Table 3—Mean intensities of several sensory attributes for 4 apple cultivars (n = 36)[a]

Cultivar/Process	Sensory attribute							
	Color	Clarity	Fruity aroma	Oxidized	Fruity flavor	Sweet	Sour	Astringent
Golden Delicious								
nonoxidative	2.6c	0.2d	2.5b	4.7a	6.1b	6.5b	3.3c	2.4c
enzyme/liquefaction	4.8a	3.1b	2.7b	6.0a	4.5c	2.7c	7.2a	5.1a
diffusion extraction	2.3c	2.6c	2.7b	5.2a	4.2c	4.8b	4.5b	3.2b
oxidative	3.4b	8.0a	4.7a	3.0b	7.1a	6.0a	4.6b	2.2c
LSD	0.5	0.6	1.1	1.5	1.0	1.1	1.2	1.2
Red Delicious								
nonoxidative	1.0d	2.4c	4.3b	5.2a	6.0a	8.7a	1.0c	1.6b
enzyme/liquefaction	7.8b	6.1b	2.7d	4.9a	6.1a	6.4c	3.3a	3.7a
diffusion extraction	9.9a	1.0d	2.9c	6.6a	5.9a	7.3b	2.0b	1.7b
oxidative	4.9c	7.3a	6.8a	2.3b	6.7a	8.8a	0.7c	0.9b
LSD	0.4	0.8	1.0	1.4	0.9	0.7	0.8	0.9
McIntosh								
nonoxidative	0.2d	0.5d	2.5c	5.3a	3.0b	2.3b	7.4b	6.9a
enzyme/liquefaction	5.2b	4.5b	3.0bc	4.1b	1.9c	1.1c	8.9a	6.5a
diffusion extraction	8.3a	2.0c	3.5b	5.3a	3.2b	3.4a	6.2c	3.9b
oxidative	3.9c	7.9a	5.1a	2.1c	5.2a	2.7ab	7.3b	4.6b
LSD	0.5	0.7	0.9	1.2	0.9	0.8	1.1	1.3
Spartan								
nonoxidative	0.8d	0.1d	3.3b	4.4b	6.3a	6.1b	3.5b	3.0a
enzyme/liquefaction	6.7	4.8b	2.2c	6.0a	4.7b	4.1c	6.9a	3.8a
diffusion extraction	9.5a	2.2c	3.3b	5.6ab	4.9b	7.8a	1.2c	1.5b
oxidative	4.7c	9.1a	6.0a	1.8c	6.6a	6.8ab	3.2b	1.8b
LSD	0.5	0.9	0.9	1.3	1.0	1.2	1.0	1.1

[a] Means within a column for a given variety followed by different letters are significantly different (LSD test, $P<0.05$).

ample, 'oxidative juices' had the lowest phenol content due to phenolic oxidation and precipitation (Lea and Timberlake, 1978). When phenolic precipitation was controlled with ascorbic acid as in the non-oxidative process highest concentrations of phenols were extracted. Diffuser extracted juice also had a high phenol content possibly due to a temperature related inactivation of polyphenoloxidase (Cumming, 1986).

Light color and high turbidity and suspended solids found in all the 'nonoxidized juices' was due to the inhibition of browning reactions. For the oxidized process, enzyme clarification coupled with precipitation of browning reaction products resulted in a juice low in suspended solids and turbidity. Also, juices extracted by enzyme liquefaction tended to be low in suspended solids and turbidity possibly due to enzymatic breakdown of pectin and other polysaccharides. With exception of 'Golden Delicious' juices, diffusion extraction produced juices with darkest colors and highest Hunterlab "a" readings indicating a hot water extraction of red pigment from the skins.

For all varieties, juice from diffusion extraction had the lowest TA and malic acid and the highest pH. This was consistent with some other studies (Wucherpfenning, 1981; Binkley and Wiley, 1978) although Cumming (1986) found no difference in TA between 'pressed juice' and 'diffuser juices'. The higher TA found in juices from enzyme liquefaction was partly explained by the galacturonic acid produced by an enzymatic hydrolysis of the pectin molecule. Those juices also had the highest malic acid content (except Golden Delicious). Possibly the cell disintegration that occurred during liquefaction of apple mash (Janda and Dörreich, 1984) increased the extraction of acids into the juices. Cell disintegration and release of cell contents may have also been responsible for the higher soluble solids found in juices extracted by enzyme liquefaction.

Process differences were also examined by sensory evaluation of visual, aromatic and flavor characteristics (Table 3). Visual assessments corresponded to analytical assessments with 'nonoxidative juice' the lightest and 'oxidative juice' the clearest. For the aromatic terms, fruity and oxidized, the former was more important since fruitiness has been associated with overall quality of an apple juice (Poll, 1981). The C-6 aldehydes including hexanal and trans-2-hexenal have been associated with fruity aroma (Panasuik et al., 1980; Dürr and Schobinger, 1981). The 'oxidative juice' had the most fruity aroma in our study while the 'nonoxidative juice' was less fruity. Results were consistent with research indicating that C-6 aldehydes are formed during crushing and pressing by an enzymatic oxidation of linoleic acid to an unstable hydroperoxide which splits to form hexanal and trans-2-hexenal (Dimick and Hoskin, 1981; Kim and Grosch, 1979).

Lower fruit aroma for the enzyme liquefication process may have been due to reduction of C-6 aldehydes to alcohols (Poll, 1988) and loss of hexanal and trans-2-hexanal (Schreier et al., 1979) during incubation with Pectinex Ultra SP. Likely the high temperature of processing combined with the length of time to extract cell contents from apple slices was responsible for the loss of fruity aroma in diffuser extracted juice.

Perception of fruit flavor intensity ('by mouth') is affected

Table 4—Overall varietal means for analytical and sensory analyses[a]

	McIntosh	Golden Delicious	Spartan	Red Delicious	LSD
Analytical (n = 8)					
TA	0.55	0.49b	0.43c	0.26d	0.01
soluble solids	11.4	12.7c	13.4b	13.7a	0.2
pH	3.37	3.49c	3.70b	0.91a	0.02
suspended solids	0.9	1.2a	1.0ab	0.7c	0.2
total phenols	630	554	538c	758a	58
settling 30	0.1	0.0c	0.0c	0.4a	0.0
settling 60	0.0	0.0b	0.0b	0.3a	0.1
turbidity	0.53	0.86a	0.69b	0.30d	0.02
Hunterlab L	59.4	60.7a	55.9c	54.3d	1.2
Hunterlab a	1.6	−2.3d	2.7b	3.6a	0.5
Hunterlab b	20.7	26.3a	25.1a	23.2b	1.4
Sensory (n = 144)					
color	4.4	3.3d	5.4b	5.9a	0.2
clarity	3.7	3.4c	4.0ba	4.2a	0.4
fruity aroma	3.5	3.2d	3.7b	4.2a	0.5
oxidized	4.2	4.7a	4.4a	4.5a	0.7
fruity flavor	3.4	5.5b	5.6b	6.2a	0.5
sweet	2.4	5.0c	6.2b	7.8a	0.5
sour	7.4	4.9b	3.7c	1.8d	0.5
astringent	5.5	3.3b	2.6c	2.0d	0.6

[a] LSD test, $p < 0.05$

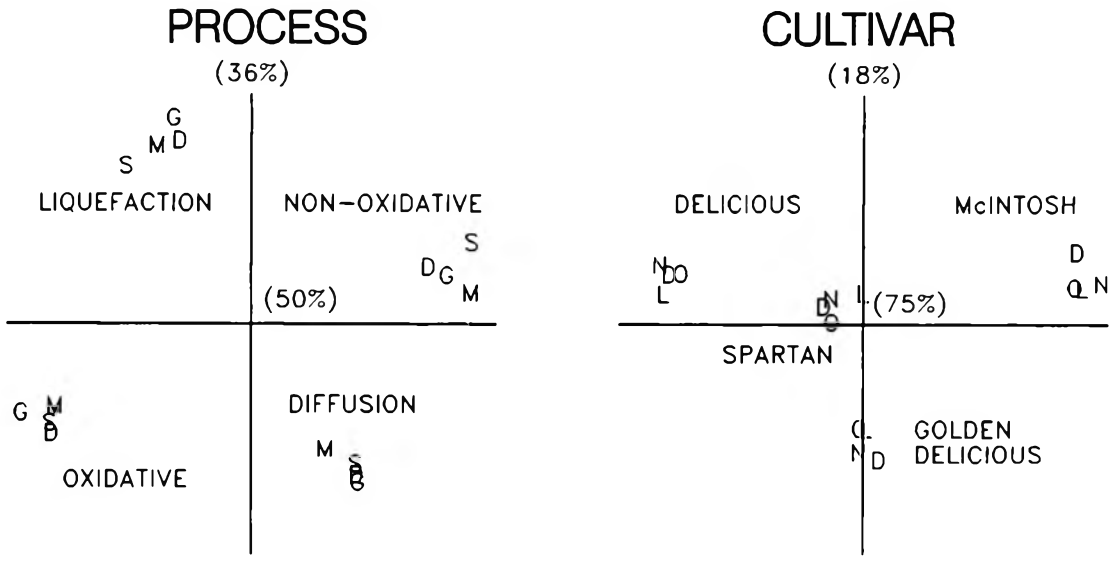

Fig. 1—Canonical discriminant analysis of analytical data using juice extraction process and cultivar for class differentiation. All juices were correctly allocated and the groups are significantly different ($p < 0.001$). S 'Spartan'; M 'McIntosh'; G 'Golden Delicious'; D 'Delicious'; L 'Liquefaction'; N 'Non-Oxidative'; O 'Oxidative'; D 'Diffusion'.

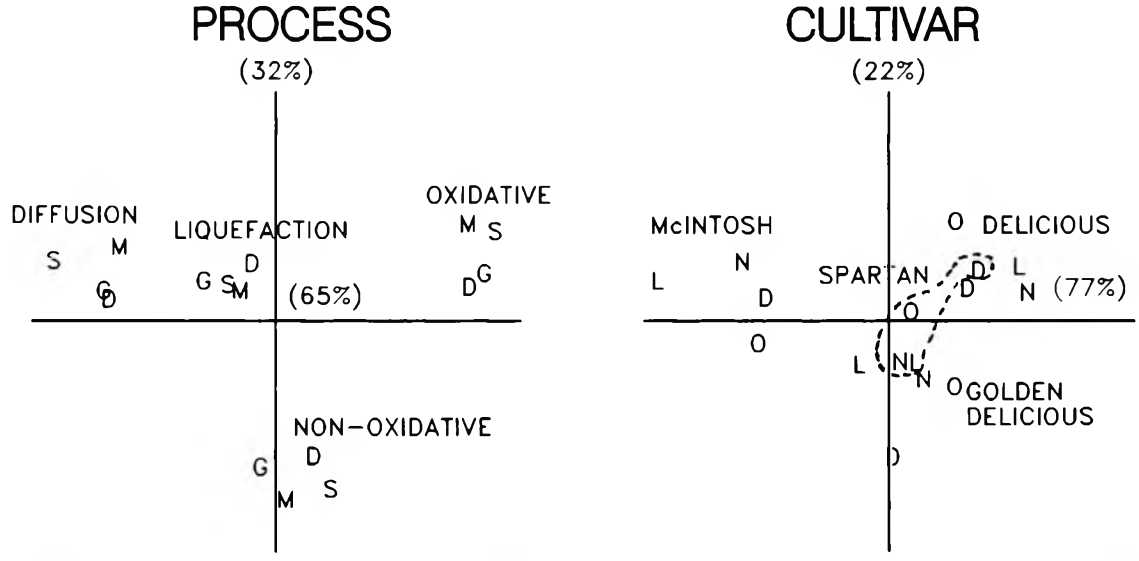

Fig. 2—Cannonical discrimant analysis of sensory data using juice extraction process and apple cultivar for class differentiation. Group separation shows that cultivar differences were not as distinct as process differences. S 'Spartan'; M 'McIntosh'; G 'Golden Delicious'; D 'Delicious'; L 'Liquefaction'; N 'Non-Oxidative'; O 'Oxidative'; D 'Diffusion'.

by other taste sensations such as sweetness (Von Sydow et al., 1974) making taste assessment more difficult. In our study the 'oxidative' extraction gave juice with highest fruit flavor. However, for the other processes, the perception of fruit flavor was influenced by cultivar. 'Delicious' juice with the highest perceived sweetness and the lowest acidity did not differ in fruit flavor intensity among processes. A high phenol content has been associated with a bitter-astringent taste (Poll, 1981). Astringency and sourness correlated ($P<0.05$) but possibly were not independent. The most sour 'enzyme liquefaction juices' were also more astringent than juices (diffuser and nonoxidative) with high phenols. Red Delicious juice with highest phenols and lowest astringency and sourness gave further evidence for sour/bitter confusion (Table 4).

Varietal characteristics, analytical and sensory, were evident in the juices (Table 4). Red Delicious juice with low acid and high soluble-solids was sweetest, least sour and most fruity (aroma and flavor). Conversely, the higher acid level and lower soluble-solids associated with 'McIntosh' apples (Strachan et al., 1951) produced the most sour and astringent juice with the least sweetness and fruit flavor. As expected, juice extracted from 'Golden Delicious' apples was light yellow without the reddish brown tones in other juices.

Canonical discriminant analyses (Fig. 1) of the significant analytical terms (ANOVA Table 2) accounted for 86% and 93% of the group differences for process type and apple cultivar, respectively. All juices were correctly allocated to process type or apple cultivar and all clusters were significantly different ($P<0.001$) from each other. This allocation confirmed analytical process and differences and indicated that unknown samples could be identified using analytical evaluations (Manly, 1986). Since the distances between the clusters is a graphic illustration of the magnitude of differences between juices, it is apparent that process type had greater impact on juice composition than did apple cultivar. Although the four processes were discriminated equally (equidistant), the four cultivars were not, with Spartan juice more similar to Red Delicious, McIntosh, and Golden Delicious juices.

Canonical discriminant analysis (Fig. 2) of mean sensory scores (Table 3), accounted for 97% and 99% of the group

—Continued on page 1627

Chitosan Coating Effect on Storability and Quality of Fresh Strawberries

AHMED EL GHAOUTH, JOSEPH ARUL, RATHY PONNAMPALAM, and MARCEL BOULET

ABSTRACT

The effect of chitosan coating (1.0 and 1.5% w/v) in controlling decay of strawberries at 13°C was investigated as compared to a fungicide, iprodione (Rovral®). Chitosan coating significantly reduced decay of berries ($P \leq 0.05$) compared to the control. There was no significant difference between chitosan and fungicide treatments up to 21 days storage. Thereafter, Rovral®-treated berries decayed at a higher rate than chitosan-coated berries. Chitosan-coated berries stored at 4°C were firmer, higher in titratable acidity, and synthesized anthocyanin at a slower rate than Rovral®-treated or nontreated berries. Chitosan coating decreased respiration rate of the berries with a greater effect at higher concentration.

INTRODUCTION

STRAWBERRY FRUIT (*Fragaria ananassa*) is highly perishable and its storage life is often terminated by fungal infection caused by *Botrytis cinerea* and *Rhizopus* sp. (Maas, 1981). The most prevalent method of maintaining quality and controlling decay of strawberries is by rapid cooling after harvest and storage at low temperatures, typically 1°C with high humidity. Since effective control of temperature during transit and storage of strawberries is difficult, other means of preservation have been sought. Because strawberries can tolerate elevated CO_2 atmosphere, they are transported in pallet-bags under high CO_2 (Bell, 1986). Although high CO_2 controls decay (El Kazzaz et al., 1983), prolonged exposure to CO_2 can cause development of off-flavors (Woodward and Topping, 1972).

Postharvest decay of strawberries can also be controlled by application of fungicides (Jordan, 1973; Aharani and Barkai-Golan, 1987). However, fungicides leave residues and the number of fungicide-tolerant postharvest pathogens is growing (Spotts and Cervantes, 1986). Thus, efforts have been made to replace fungicides by natural products or to intensify natural defenses of the tissue to control decay and prolong storage life (Adikaram et al., 1988; Boulet et al., 1989).

Recently, semi-permeable coatings have been advanced to improve storability of perishable crops (Lowing and Cutts, 1982). Application of «Pro-Long» (a blend of sucrose esters of fatty acids and sodium carboxymethyl cellulose) to bananas delayed ripening by modifying the internal atmospheres (Banks, 1984). Delay of ripening was also reported in pears and apples coated with «Nutri-Save®» (N,O-carboxymethyl chitosan) (Elson et al., 1985; Davis et al., 1988). However, for strawberries coating with semi-permeable film has not been explored.

Chitosan, a high molecular weight cationic polysaccharide, theoretically should be an ideal preservative coating material for strawberries. It has been shown to inhibit growth of several fungi (Allan and Hadwiger, 1979; Stössel and Leuba, 1984; El Ghaouth et al., 1989; Hirano and Nagao, 1989), to induce chitinase, a defense enzyme (Mauch et al., 1984) and to elicit phytoalexin (pisatin) in pea pods (Hadwiger and Beckman, 1980; Kendra and Hadwiger, 1984). Due to its ability to form semi-permeable film (Bai et al., 1988), chitosan coating can be expected to modify the internal atmosphere as well as decrease the transpiration losses. Therefore a delay in ripening and control of decay by means of chitosan coating could result. Chitosan is nontoxic (Arai et al., 1968), and its biological safety has been recently demonstrated by feeding trials with domestic animals (Hirano et al., 1990). The objective of our study was to assess the potential of chitosan coating as an antifungal agent compared to a fungicide, iprodione (Rovral®), in controlling decay of postharvest strawberries, and to determine the effect of chitosan coating on quality and storability.

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MATERIALS & METHODS

Fruits and chemicals

Strawberries (*Fragaria ananassa* Duch. cv Kent) grown in local farms were harvested and immediately cooled with vapors of dry ice. The berries of uniform size with 50% or less red color, free of physical damage and fungal infection were used. The berries were randomly distributed into groups of 70 fruit. Each group represented one replicate, and for each treatment four replicates were used. Crab-shell chitosan was purchased from ICN Inc. (Cleveland, OH). Iprodione, (Rovral®) was obtained from May and Baker Canada Inc. All other chemicals were of analytical grade.

Chitosan coating solutions

To prepare 100 mL of chitosan solutions (1.0 and 1.5%) w/v), 1.0 or 1.5g of chitosan was dispersed in 80 mL of sterile deionized water to which 2.5 mL of 10N HCl was added to dissolve the chitosan. The pH of the solution was adjusted to 5.6 with 1N NaOH, and 0.1 mL of Tween 80 was added to the solution to improve wettability. The solution was then made up to 100 mL. An acid solution containing Tween 80 without chitosan, pH 5.6, was used as control.

Preparation of inoculum

Botrytis cinerea was isolated from infected strawberries and maintained on potato dextrose agar (PDA). Conidia of *B. cinerea* were recovered by filtering the mycelial suspension of 2-wk old culture through 3 layers of sterile cheese cloth. The concentration of the conidial suspension was adjusted to 2×10^5 conidia per mL.

Decay control

Strawberries were immersed in a conidial suspension of *B. cinerea* containing 0.1% (v/v) Tween 80, and allowed to air dry at room temperature for 2 h. Inoculated berries were individually dipped in aqueous suspension of Rovral® (100 μg/mL) or in chitosan solutions (1.0 and 1.5% w/v) or in water control. Four replicates of 70 berries were used for each treatment. After drying, the berries were stored at 13°C in containers continuously ventilated with humidified (95% RH) air at 20 L/hr. Berries were examined for mold on alternating days and a berry was considered infected when a visible lesion was observed. The results were expressed as percentage of berries infected. The results were subjected to Analysis of Variance (ANOVA) with 5% LSD values calculated to separate significantly different means of the treatments.

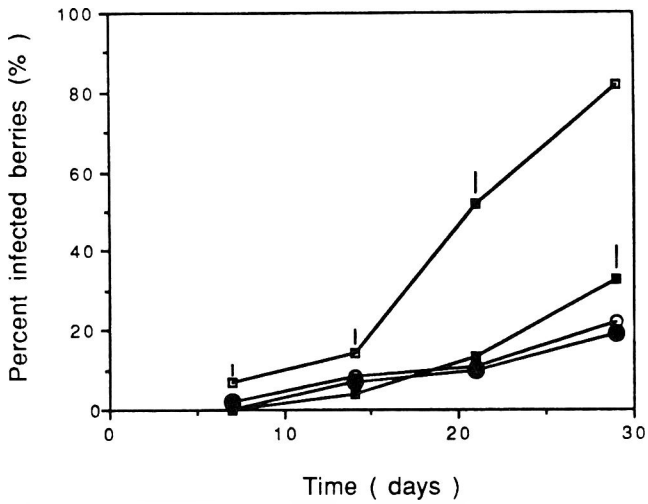

Fig. 1—Effect of chitosan coating, 1.0% (○), 1.5% (●), and Rovral® (iprodione) treatment (■) on the decay control of strawberries stored at 13°C compared to the control (water-treated) berries (□). Means of four replicates. Vertical bars represent LSD at 5% level among treatments.

Quality attributes

The effect of chitosan coating and Rovral® on quality was assessed separately with non-inoculated berries. They were dipped in Rovral® suspension (100 μg/mL) or in chitosan solutions (1.0 and 1.5% w/v) or in water control and stored at 4°C as described. All treatments contained 0.1% Tween 80. Each treatment had 4 replicates, and each replicate consisted of 70 berries. Quality of the berries was assessed each week. A sample of 15-20 berries in total was randomly removed from each treatment and analyzed for firmness, titratable acidity and anthocyanin content. To determine firmness, the berries were sliced into halves and each half was punch tested. The penetration force (Newton) of the flesh was measured with an Instron Universal Testing Machine (Model 1101, Instron Corp., Canton, MA) using a 4 mm flat plunger (Holt, 1970). Titratable acidity was expressed as mg of citric acid/100 mL. Acidity was determined using 10 g aliquot of purée in 40 mL deionized water and titrating with 0.1 N NaOH to an end-point of pH 8.1. Anthocyanins were extracted with acidified ethanol from a 2 g aliquot of a homogenate of 7 berries, according to the method of Fuleki and Francis (1968). Anthocyanin content was expressed as mg anthocyanin/100g strawberry homogenate. The quality evaluation data were subjected to Analysis of Variance (ANOVA).

Respiration

The respiration rate of strawberries at 4°C was determined by placing them (120 g) in an air tight container (1.35-L) for 2 to 4 hr. Then a 5 mL gas sample was withdrawn with a gas tight hypodermic syringe and analyzed for CO_2 using a gas chromatograph equipped with a TCD detector and a Poropak N Column. Four replicates of each treatment were analyzed.

RESULTS & DISCUSSION

Antifungal effect of chitosan coating and Rovral®

Decay of strawberries was reduced significantly ($P \leq 0.05$) when inoculated-berries were either coated with chitosan or dipped in Rovral® (Fig. 1). The early signs of mold development in the strawberries, regardless of the treatment, appeared after 8 days storage at 13°C. After 21 days at 13°C, the percentages of decayed-berries in chitosan-coated (1.0 and 1.5% w/v) and Rovral®-treated were 11, 10 and 13% respectively, while in the control it was 52%. There was no significant difference between chitosan and Rovral® treatment in controlling decay before 21 d storage. Thereafter, however, the Rovral®-treated berries decayed at a slightly higher rate than the chitosan-coated berries suggesting the active ingredient of the fungicide could have been inactivated by the host. Furthermore, Rovral®-treated berries showed symptoms of phytotoxicity characterized by formation of water-soaked areas. At the end of storage (29 days), decayed berries in control was about 82% and in Rovral® treatment 33%. In contrast, the level of decay in 1.0 and 1.5% chitosan-coated berries was 22 and 19%, respectively. There was no added benefit to decay control by increasing concentration of chitosan from 1.0 to 1.5%.

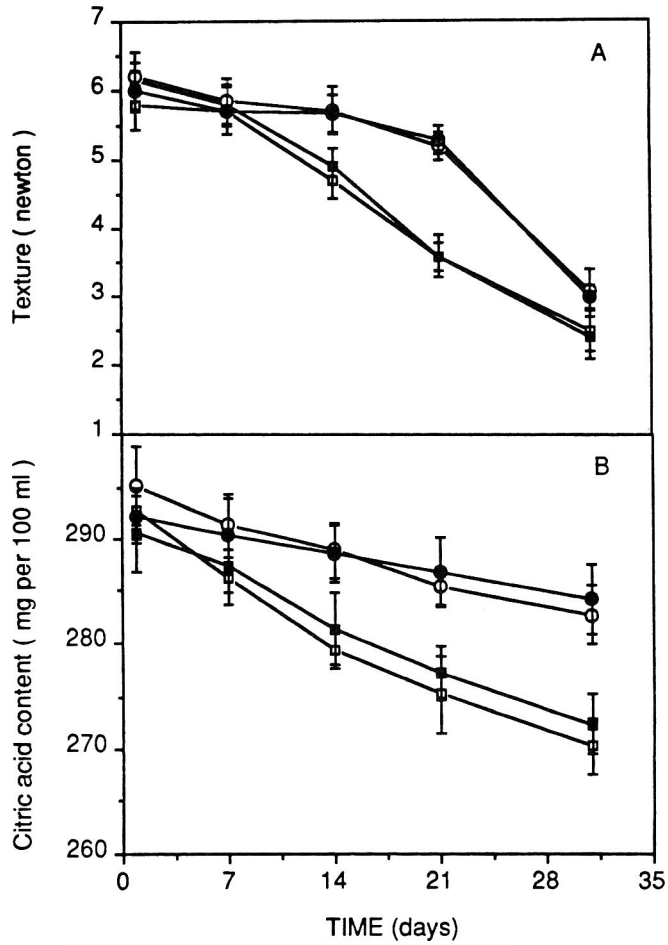

Fig. 2—Texture (A) and Titratable acidity (B) of control (□), Rovral®-treated (■), 1.0%, (○) and 1.5% (●) chitosan-coated strawberries stored at 4°C. Vertical bars represent SE values.

Chitosan has the capacity to inhibit growth of several fungi, to induce chitinase, and to elicit phytoalexins in the host tissues. Thus, the control of decay in strawberries could be attributed to either the fungistatic property of chitosan per se or to its ability to induce defense enzymes (i.e. chitinase and β-1,3-glucanase) and phytoalexins in plants or a combination. Whatever the mode of action, chitosan proved more effective than Rovral® in controlling decay of strawberries at 13°C.

Effect on quality attributes

Chitosan coating had a beneficial effect on flesh firmness, titratable acidity and retarding synthesis of anthocyanin of strawberries stored at 4°C (Fig. 2 and 3). Those coated with chitosan (1.0 and 1.5%), after 31 d were firmer and higher in titratable acidity than the control or Rovral®-treated berries (Fig. 2). Increasing chitosan concentration did not result in any increase in retention of firmness or modify titratable acidity. Rovral® treatment was effective in controlling decay, but did not improve firmness or acidity as compared to the control.

Chitosan coating delayed rate of ripening as indicated by anthocyanin content (Fig. 3). The total anthocyanin content of chitosan-coated berries was the least among the treatments, after 31 days storage. In addition, the anthocyanin content of Rovral®-treated berries was greater than that of the control.

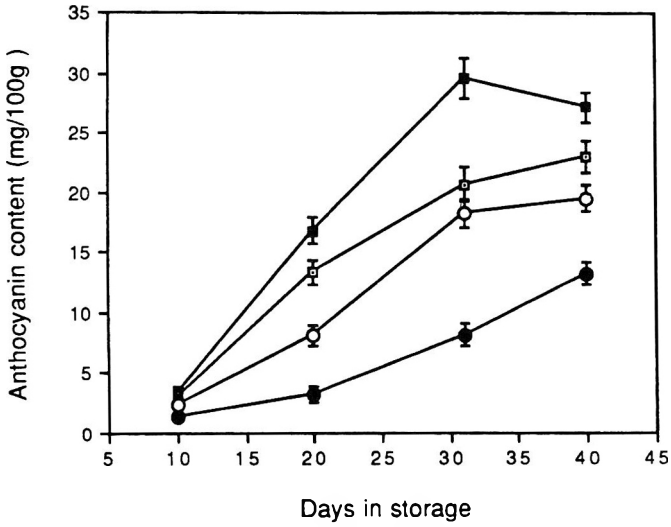

Fig. 3—Anthocyanin content of control (□), Rovral®-treated (■), 1.0% (○) and 1.5% (●) chitosan-coated strawberries stored at 4°C. Vertical bars represent SE values.

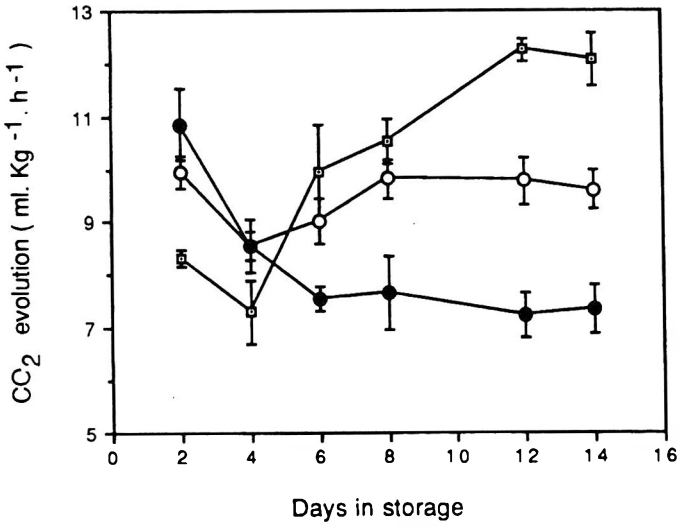

Fig. 4—CO_2 production of control (□), 1.0% (○) and 1.5% (●) chitosan-coated strawberries stored at 4°C. Vertical bars represent SE values.

This suggested that Rovral® treatment may have stimulated the ripening process. On the contrary, chitosan-coated berries synthesized anthocyanin at a slower rate, and coating neither affected appearance nor caused apparent phytotoxicity. Furthermore, chitosan-coated berries developed the full red color after 35 days storage. Retention of firmness, higher titratable acidity and slower rates of anthocyanin production in coated berries demonstrated that chitosan coating slowed down metabolism and prolonged the storage life. Coating fruits with semi-permeable film has generally been shown to retard ripening by modifying the endogenous CO_2, O_2 and ethylene levels of fruits (Lowings and Cutts, 1982). However, specific instances of interference with normal fruit ripening have been reported with «Pro-Long» and «Nutri-Save®» (Meheriuk and Lau, 1988). While available coating materials have been found to extend storage-life of produce by acting as a diffusion barrier, chitosan coating affords the added advantage of antifungal activity which is beneficial for highly perishable produce such as strawberries. In addition chitosan coating is likely to modify the internal atmosphere without causing anaerobic respiration, since chitosan films are more selectively permeable to O_2 than to CO_2 (Bai et al., 1988).

Effect of chitosan coating on respiration

The CO_2 production of strawberries stored at 4°C is presented in Fig. 4. The pattern of respiration for the 1.5% samples was different than those of the control and 1.0% samples. However, coating with chitosan had an immediate stimulatory effect on respiration. Such effects gradually disappeared during the following 2 days. Reduction of respiration rate by chitosan coating became evident beyond the 4th d of storage. The effect of chitosan coating on CO_2 production was greater at higher concentration.

CONCLUSIONS

CHITOSAN COATING was more effective than Rovral® treatment in controlling postharvest decay of strawberries at 13°C. Chitosan-coated berries stored at 4°C were firmer and higher in titratable acidity than Rovral®-treated or control berries. Chitosan coating neither altered ripening capacity of strawberries nor caused any apparent phytotoxicity. Our study indicated that preservative coating with chitosan has a potential to prolong storage life and control decay of strawberries even at higher storage temperatures. In addition chitosan coating with its ability to modify internal atmosphere in the tissue and fungistatic property can provide a security factor when rigorous control of storage and distribution temperatures cannot be assured. However, the organoleptic quality of the chitosan coated fruits remains to be evaluated.

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Pectin Concentration, Molecular Weight and Degree of Esterification: Influence on Volatile Composition and Sensory Characteristics of Strawberry Jam

E. GUICHARD, S. ISSANCHOU, A. DESCOURVIERES and P. ETIEVANT

ABSTRACT

Composition of headspace, consistency, taste and flavor characteristics were determined in jams made with different pectins. At usual concentrations, high methoxylated pectin induced an undesirable modification of typical flavor and intensity of flavor and taste, whereas low methoxylated pectin induced few alterations. At fixed concentration and molecular weight, a decrease in degree of esterification produced a significant decrease in consistency and noticeable modifications of the flavor perception and headspace composition, but no taste alteration. Mechanical reduction of pectin molecular weight significantly modified only the consistency.

Key Words: strawberry, esterification, molecular-weight pectin, volatiles

INTRODUCTION

GELLING AGENTS are added to commercial products to achieve desired firmness or consistency (Doublier and Thibault, 1984). Among them, pectic substances find many applications particularly in jam manufacturing. Pectin is a naturally occurring polysaccharide, mainly extracted from citrus peel and apple pomace. High methoxylated pectins (HMP) are used to form gels in acidic media of high sugar content (Leroux and Shubert, 1983), and low methoxylated pectins (LMP) are used in products of lower sugar content (Thibault and Petit, 1979). The strength of gels obtained with LMP varies essentially with concentration of calcium ions in the medium but also with the molecular characteristics of the polysaccharide. In model systems, Pilnik (1970) showed that, at pH <3, viscosity increased while degree of esterification of HMP decreased. At a specific degree of methylation, the physical properties of a HMP are modified by the dispersion and location of remaining free carboxylic groups (Crandall and Wicker, 1986). The molecular weight of pectin can also influence some gel strength characteristics. Crandall and Wicker (1986) found that the elasticity modulus was influenced primarily by the short stiff chains and was independent of pectin molecular weight (MW). On the contrary, they also concluded that breaking strength was influenced primarily by the longer, more flexible chains which remained cross linked after the short stiff chains had ruptured, (which were related to MW), Panchev et al. (1988) tested different pectins and found that the optimal strength of the gel corresponded to a degree of esterification (DE) of 57–58%. However, they tested pectins in which MW was also decreasing with degree of esterification, thus precluding a strict conclusion about the relation between gel strength and DE. In fact, gel strength is also related to molecular weight of pectins as shown by Gregory (1986) in dairy products when testing usual or Mex pectins (pectins with MW>100,000). These reported discrepancies probably reflect difficulties in determining accurate molecular weight values for pectin.

Many studies have demonstrated that hydrocolloids not only modified viscosity, but often reduced intensities of odor, taste and flavor (e.g. Moskowitz and Arabie, 1970; Pangborn et al., 1973). Some evidence indicated this masking effect varied with type and concentration of hydrocolloid used. Most of these studies, such as that by Marshall and Vaisey (1972), concerned the effect of hydrocolloids on taste qualities in model solutions. Nevertheless, Lundgren et al. (1986) studied the effect of pectin on odor, taste and flavor intensities in jams, but at concentrations 10 times higher than those used in jam manufacturing. The objective of our study was to clarify the influence of the amount of pectin added, and the DE and MW of that pectin on sensory characteristics (such as consistency of the gel, typical flavor and intensity of flavor), and on amounts of volatile compounds in headspace.

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MATERIALS & METHODS

Pectin preparation

One rapid set HMP and one LMP from Mero Rousselot Satia (France) were used. Characteristics are listed in Table 1. Both were nonstandardized citrus pectins (245°SAG for the HMP), currently recommended for standard jam manufacturing (60°Brix, 45% fruit).

Degree of esterification and molecular weight. DE was measured by titration of acidic functions of the galacturonic monomers before and after saponification as recommended by Unipectine (Mero Rousselot Satia). MW was determined from pectin solutions (300 to 600 $mg.L^{-1}$ in 155 mM NaCl) according to Owens et al. (1946), using an Ostwald's capillary viscometer maintained in a 25°C thermostated bath, by extrapolating to zero concentration.

Pectin deesterification. 100 mL of a pectin methyl-esterase (Rapidase CPE from *Aspergillus Niger*, 88 $UI.mL^{-1}$), free of polygalacturonase and of lyase (Baron et al., 1988) was added to 100 mL of a 2 $g.L^{-1}$ HMP solution maintained at 30°C (pH 4.5). Production of galacturonic acid groups was continuously neutralized by addition of NaOH (0.1N) to maintain the original pH. Enzymatic reactions were stopped by precipitation of the pectin and of the enzyme with addition of 30 mL of an aqueous copper acetate solution (7%). The coagulum was then washed with an acidic ethanolic solution cooled to 4°C (80% ethyl alcohol and 1% HCL) until no more copper remained, and then with an ethanolic solution (70%) to remove chloride ions. The purified pectins were then washed with diethyl ether, dried in a desiccator one night under vacuum and stored there with P_2O_5. Pectins of predetermined DE were thus obtained by stopping the reaction when the volume of NaOH corresponded to neutralization of the number of carboxylic groups theoretically released. Respectively 10 mL of NaOH were added for pectins with 66 DE and 19.7 mL for 54 DE (instead of 62 DE and 50 DE expected).

Mechanical degradation of pectin. Three batches of the same HMP were mechanically milled with ceramic cylinders in a ceramic pot for 3, 6 or 12 hr (Van Deventer-Schriemer et al., 1987).

Jam manufacturing

Deep-frozen berries (2 kg) were added in a 6 L preserving pan, containing 250 mL boiling deionised water (Millipore). When the temperature reached 90°C, 2.2 kg sugar was added, and the mixture was continuously stirred while heating. The pectin, diluted in 400 mL, was added as soon as the mixture reached 65°C Brix. Heating was stopped at 59° Brix, and 20 mL of citric acid solution (0.5 $g.mL^{-1}$) was added. Jam was immediately poured in jars. For sensory analysis, a homogeneous medium was prepared by crushing the jam in a co-

Table 1—Characteristics of the pectins and of the strawberry jam samples

Experiment	Strawberries: Cultivar	Origin	Preparation of jams: Fruit kg	Sugar kg	Type	DE[a]	Pectin MW[b]	Methoxy-groups	DA[c]	Chemical parameters of jams: Soluble solids °Brix	pH
H	Senga sengana	Poland	2.0	2.2	HM	83	86000	9	–	60	3.10 ± 0.01
L	Senga sengana	Poland	2.0	2.2	LM345	37	59000	6	12	60	3.20 ± 0.01
HDE	blended	France	2.0	2.2	HM	83	86000	9	–	60	3.08 ± 0.02
						66	86000	11			
						54	66000	9			
HMW	blended	France	2.0	2.2	HM	83	86000	9	–	60	3.15 ± 0.05
						83	75000	9			
						83	59000	9			
						83	32000	9			

[a] DE = degree of esterification.
[b] MW = molecular weight.
[c] DA = degree of amidation.

lander with a pestle. After cooling to room temperature, final pH was measured (Tacussel pH-meter, TCBC 17/15 probe). All preparations had a final pH of 3.10 ± 0.1 and 60° Brix.

Experimental samples

Four experiments were carried out (Table 1). Experiment H consisted of a preparation of 5 jams containing increasing percentages of a HMP (0, 0.05, 0.1, 0.2 and 0.4%) and experiment L, a LMP (0,0.1, 0.2, 0.4 and 0.6%). Experiment HDE was conducted to study effect of 0.2% HMP with varying DE and experiment HMW to study effects of MW on flavor and texture perception of jam. In each, a control sample without added pectin was included.

Chemical analysis

Isolation of volatiles. A headspace analysis was used in order to avoid gel disruption. Four hundred grams of jam were introduced into a 1L flask and extracted following the method described by Rapp and Knipser (1980) and modified by Guichard and Ducruet (1984). The vapor phase was stripped 19 hr by a stream of 110 mL.min^{-1} purified nitrogen and volatile compounds were trapped in a liquid-liquid continuous extractor containing 250 mL of a 10% ethanolic solution. This solution was continuously extracted with freshly distilled dichloromethane (100 mL) and solvent vapors were condensed at −30°C. Each analysis was done in duplicate. For quantification of volatiles, n-tridecane (25 pg/g of jam) was added as internal standard in the solvent extract. Analyses were performed after concentration to 100 mL using Kuderna-Danisch apparatus (Garnier, Quetigny, France) of appropriate sizes.

Gas chromatography. Gas-chromatographic analyses of the extracts were performed using a Girdel 300 gas chromatograph equipped with a chemically bonded DB5 fused silica capillary column (30m, 0.32 mm i.d., 1 μm, J & W, Scientific Inc.). Injection temperature was 220°C and detector, 250°C and extracts (1μL) were injected splitless. After injection, oven temperature was held at 30°C for 5 min and then programmed at 2°C.min^{-1} to 220°C. The flow rate of the carrier gas (H_2) was 37 cm.sec^{-1}. For quantification, an Enica 10 integrator (Delsi France) was used. Odors of compounds eluting from the column were assessed by 3 judges, as described by Etievant et al. (1983).

Gas-chromatography-Mass-spectrometry. Identifications were determined on each extract using a Nermag R 10-10/C mass spectrometer coupled with the same gas chromatograph equipped with a DB5 column (60 m, 0.32 mm i.d;, 1 μm, J & W, Scientific Inc.). Ionization was by electronic impact at 70 eV.

Data analysis. For each experiment, a univariate analysis of variance was performed using the SAS statistical package (SAS/STAT$_{TM}$ 1987). Only curves showing significant variations ($p<0.05$) of volatile amounts between samples were considered.

Sensory analysis

Subjects. Candidates were recruited by advertisements to participate in a large number of sensory evaluations on strawberry jams. They were selected on ability to memorize and recognize basic tastes and odors and to rank jams with different pectin levels on oral consistency. Eighteen subjects, 8 males and 10 females, 18 to 45 years old, were selected and at least 12 were present at any session. Tasters were paid and tests were completed within 6 mo. During this time, they were not informed of the aim of the study.

Flavor description. Quantitative descriptive analysis (Stone et al., 1974) was performed on the jams. During 4 sessions, descriptive terms were generated by the judges from individual evaluation of commercial strawberry jams, and from those made in experiment H. After each session, they were encouraged to discuss any descriptor which they felt adequately described a jam's flavor. A list of 25 terms was then established by the experimenter. During a fifth session, panelists rated the intensity of each term using an unstructured, 13 cm scale, for each jam from experiment H. Results were then discussed and the most appropriate terms used to define flavor differences among the jams were selected for the final list of 10 flavor attributes (total intensity, typical flavor, fresh strawberry, unripe strawberry, overripe strawberry, cooked strawberry, candied fruit, caramel, artificial, lemon) and of 3 gustative attributes (sweetness, acidity and sharpness).

Evaluation of consistency. At the beginning of the study, before objective measurements, the most preferred level of jam consistency was estimated for each member of the panel. The samples were the five jams of experiment H. There were two sessions and at each session, all five pectin concentrations were presented in random order. The scale used was a 10 cm graphic rating of ideal-relative intensity as proposed by Booth et al. (1983). This indicated "just right" in the center with anchors of "not hard enough" and "too hard" at the ends. Evaluation of consistency was made separately by the same group of subjects. Oral consistency was rated using an unstructured, 13 cm scale, with a verbal anchor point at each end (left anchor = very soft; right anchor = very hard), as described (Issanchou et al. 1990).

Evaluation conditions. Evaluations were conducted in individual air conditioned (20 ± 0.5°C) booth. Red lights were used to mask possible color differences. One sample was evaluated at a time. Judges rinsed with mineral water between samples; bread was also available. About 20g of each jam was served in white plastic beakers coded with 2 or 3 digit numbers. The samples of each experiment were presented within 1 or 2 sessions. Some, or all samples were presented twice, in random order. The analysis took place within 12 wk after samples had been prepared. Responses for quantitative descriptive analysis were digitized for statistical analysis by measuring, in mm, the segment length from left of the scale to the judge's mark (from 0 to 130).

Data analysis. For each experiment, a two-way analysis of variance with interaction (sample x subject) was performed using SAS. If the sample effect was significant, the t-Bonferoni method was used at the 5% level to make pairwise comparisons between sample means. The mean intensity ratings were plotted only for terms with a significant sample effect ($p<0.05$). In order to determine the best jam consistency for each subject, the individual ideal-relative intensity ratings were plotted on pectin concentration. Linear regression was calculated by the least squares method for each subject and the ideal pectin concentration corresponding to "just right" was calculated.

RESULTS

Volatile compounds identified in the jams

Table 2 gives the names and odors of the compounds identified in jams without pectin with three different batches of

Table 2—Volatile compounds identified in the jams and their odor evaluation

No.	Compounds	Kovats[b] index	Odor evaluation
1	3-methylbutanal[a]	600	
2	3-methyl-2-butanone	607	
3	3-methyl-3-pentanol[a]	620	
4	2-pentanone	640	fruity, banana
5	pentanal	651	slightly fruity, herbaceous, nut-like
6	2-pentanol	677	fruity
7	3 hydroxy-2-butanone	685	butter
8	ethyl propanoate	691	fruity, apple, banana
9	methyl butanoate	705	fruity
10	2-methyl-2-pentanol[a]	712	
11	1-pentanol	717	chemical, nauseating
12	3-penten-2-one	724	herbaceous
13	2-hexanone	771	sweet, fruity
14	3 ethoxypropanal[a]	786	butter
15	butyric acid	790	cheese
16	hexanal	800	freshly cut grass
17	ethyl butanoate	805	apricot
18	2-hexanol	817	butter
19	butyl acetate	824	flowery
20	furfural	828	pungent, caramel
21	*trans*-2-hexenal	850	green-fruity, fresh-green
22	3-methyl-2-hexanol[a]	859	
23	1-hexanol	870	chemical, winey, slightly fatty, fruity
24	5-methyl-2-hexanol[a]	878	rubber
25	2-heptanone	893	flowery, nectarine
26	heptanal	901	oily, fatty, rancid
27	2-acetylfuran	907	herbaceous, balsamic, sweet
28	methyl hexanoate	924	fruity
29	2-methylpropyl butanoate	928	
30	benzaldehyde	963	butter, almond
31	4-ethylgaiacol	972	clove
32	hexanoic acid	990	cheese, fatt, -rancid
33	ethyl hexanoate	1000	fruity
34	octanal	1005	potato
35	hexyl acetate	1013	fruity
36	furan	1026	spicy, smokey
37	mesifurane	1060	caramel
38	acetophenone	1071	herbaceous
39	heptanoic acid	1081	unpleasant, sour, sweet-like
40	linalool	1103	flower of orange
41	nonanal	1106	fruity, flowery
42	3-nonen-2-one	1141	herbaceous
43	octanoic acid	1163	cheese
44	α-terpineol	1185	herbaceous, green
45	decanal	1188	cooked
46	2-methylhexyl-butanoate	1194	jasmine
47	geraniol	1250	flowery
48	nonanoic acid	1252	nut
49	decanoic acid	1349	unpleasant
50	ethyl decanoate	1379	flowery
51	dodecanoic acid	1544	butter
52	nerolidol	1552	peanut

[a] Identified in strawberry for the first time but previously reported as natural compound. Mass spectrum identical to that published (NPS/EPA/NIH).

[b] Calculated on the DB5 column by simultaneous injection of hydrocarbons from C_6 to C_{18}.

strawberries. The amounts of several of these compounds varied considerably according to batch of fruit. These variations were particularly notable for 1-pentanol [11] (1.68 to 62.9 ppm), 2-methyl propyl butanoate [29] (0 to 71.6 ppm), hexanoic acid [32] (0.24 to 78.8 ppm), octanal [34] (4 to 40 ppm), octanoic acid [43] (0.88 to 16.8 ppm) and 2-heptanone [25] (0.6 to 10.54 ppm). Such differences may explain the different odor intensities and qualities of jams made with these different batches of fruits. However, they indicated it was not possible to compare results from one experiment to the other.

Ideal consistency

Regressions calculated for each of the 18 assessors were statistically significant ($p < 0.01$). The histogram of the ideal values (Fig. 1) showed that only one subject preferred jam with a HMP concentration higher than 0.2%. The mean ideal consistency was calculated to correspond to HMP concentration near 0.11%. This value seemed low compared to amounts currently used in jams (0.2%) but since the HMP was not standardized, the corresponding amount of standardized pectin (150° SAG instead of 245) should be 0.18%.

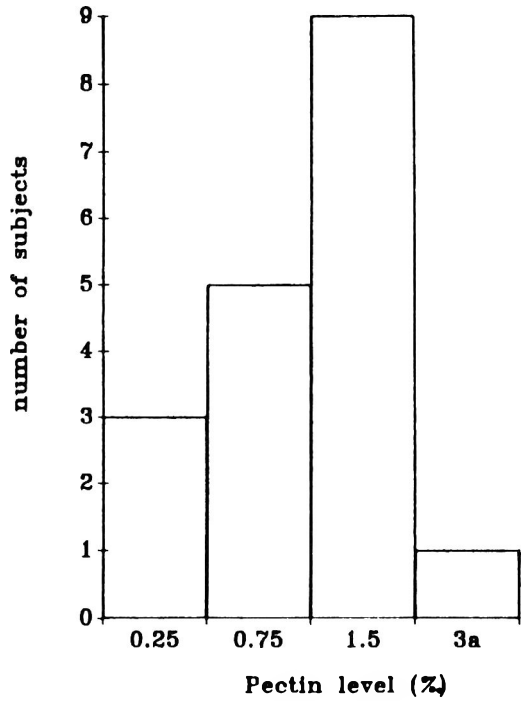

Fig. 1—Histogram of the ideal consistency values of jam. (Pectin level is the midpoint for each category.)

Influence of amount of HM pectin on jam (Experiment H)

Increasing the amount of added pectin increased the oral consistency of the jam (Fig. 2a). On the contrary, sweetness, acidity and sharpness decreased with increasing amounts of pectin. Fig. 2b shows that the overall intensity and typical note of the flavor of the jam decreased consequently. The same figure shows a decrease of strawberry and caramel notes and an increase of the candied note, which may explain the decrease in the overall typical flavor. Headspace analysis showed that only seven of the compounds analysed for were significantly affected by an increase in pectin. Figure 2c shows that adding only 0.05% of pectin decreased drastically the amount of ethyl hexanoate [33], and to a lesser extent that of the other compounds. The decrease in the head-space of mesifurane [37], a compound described as caramel-like in Table 1, could explain a part of the decrease of the caramel note in the jam, and also the decrease of the typical flavor of the jam (Fig. 2b and 2c). Moreover, the amounts of some esters such as butyl acetate [19], ethyl hexanoate [33] and hexyl acetate [35], (compounds exhibiting flowery or fruity notes) (Table 2), and that of aldehydes such as benzaldehyde [30], nonanal [41] and trans 2-hexenal [21], (almond and green odors), decreased with the typical flavor of the jam's aroma, from 0 to 0.2% of pectin. The low increase in amounts of these compounds in the jam prepared with 0.4% of pectin could be explained by the greater exchange area in the flask during headspace analysis. When the jam was poured into the flask, it was too thick to spread out. Since Doudlard and Guichard (1989, 1990) demonstrated the contribution of these compounds to strawberry aroma, the variations of concentration could be directly responsible for the modifications in aroma.

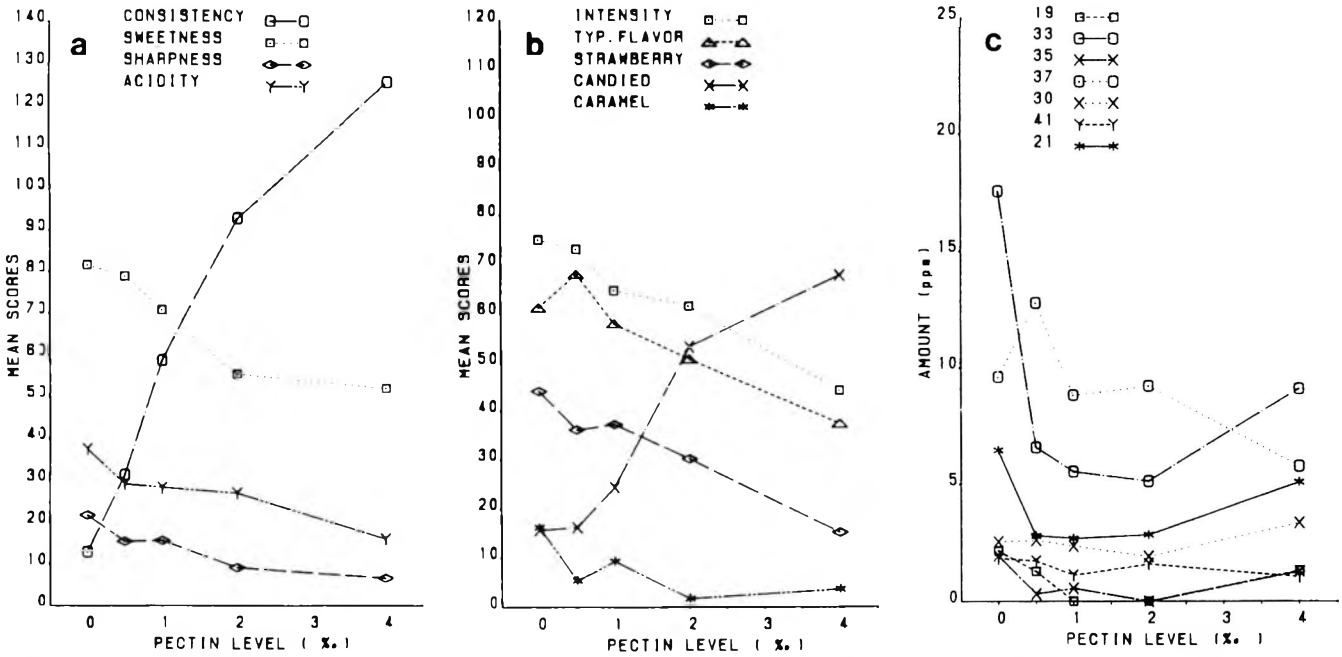

Fig. 2—Effect of HM pectin level: a, b: on sensory characteristics; c: on volatile composition in head-space. (Numbers refer to Table 2).

Influence of the amount of LM pectin on jam (Experiment L)

As previously noticed with HMP, oral consistency increased with increasing amount of added pectin (Fig. 3a). However, a comparison of Fig. 2a and of Fig. 3a shows that a consistency of 100 was obtained with about 0.25% of HMP vs. 0.6% of LMP, using the same batch of fruit and the same sugar level (60°Brix). Acidity was not reduced by addition of pectin and sharpness was reduced only when 0.6% pectin was added. Sweetness was also affected at the same level of addition, but much less than when 0.2% of HMP was added, which corresponded to the same consistency.

The sensory assessment of the jam made with different amounts of LMP did not show significant differences for typical flavor and intensity of the aroma. As shown in Fig. 3b, the cooked fruit note varied independently of the amount of pectin and the candied fruit note was the only flavor characteristic which increased with it, although to a lesser extent than when using HMP. In the headspace, a regular increase of concentration of such esters as methyl- and ethyl butanoates [9, 17], methyl- and ethyl hexanoates [28, 33] and hexyl acetate [35], which possess fruity notes, and of 3-penten 2-one [12], which is described as herbaceous, could be observed (Fig. 3c). These changes in amounts seemed not to affect the overall perception of aroma. Nevertheless, it was notable that volatiles increased when LMP was added instead of the decrease observed when HMP was added. The increase of the candied note could not be explained by the increase of these substances. It was more probably due to the increase of some other volatile compounds which we did not detect.

Influence of degree of esterification (Experiment HDE)

Figure 4a shows a significant decrease of oral consistency when DE was enzymatically decreased from 83 to 66. No significant difference was observed when DE was further decreased to 54, but the molecular weight of the pectin was also modified in this last sample (Table 1). As in experiment H, the addition of 0.2% of the original pectin in jam induced a decrease in sweetness and acidity. However the slight increase in these characteristics (Fig. 4a), corresponding to a decrease in DE, was not significant. Aroma characteristics differed between the control and the jams in which the original pectin were added (Fig. 4b). As before, the overall intensity of aroma, the cooked fruit and the caramel notes were found to increase while the candied fruit note decreased. However, decreasing the DE of the pectin seemed to restore the original intensities of overall aroma and its characteristics.

Concentrations of many volatile compounds in the jam were affected by modification of DE. The amounts of volatile compounds which exhibited significant variations between the jams are reported in Fig. 4c, 4d, and 4e. The amounts of benzaldehyde [30], octanal [34], nonanal [41], methyl- and ethyl butanoates [9, 17] and hexanoic acid [32] were lower in the head-space of jam made with original pectin. This could explain the lower aroma intensity perceived in that sample, compared to the jam made without pectin. The higher aroma intensity reported when DE was decreased from 83 to 54 could also be related to the increase in amounts of benzaldehyde, nonanal, methyl and ethyl butanoates to a level near that found in the jam without pectin. The only compound which decreased with DE was 3-methylbutanal [1]. This could not be responsible for the parallel decrease of the candied note, which must therefore be due to a trace volatile constituent. On the contrary, the higher caramel and cooked notes found when using low DE pectins could be explained by higher furfural [20] (Table 2).

Influence of the molecular weight (Experiment HMW)

Figure 5a shows a large difference in consistency of jams made with the initial pectin (MW = 86,000) and those made with the pectin milled for 6 and 12 hr, but little difference was observed between the two latter samples. Compared to consistency, sweetness was only slightly affected by MW reduction. For aroma, a more complex pattern was observed (Fig. 5b). As in the other experiments, addition of original pectin caused an increase in candied fruit note and a decrease in caramel note. Moreover, fresh strawberry note was lower in the reference as opposed to the overripe strawberry note.

Volatile aromatic compounds, particularly esters, ketones and some aldehydes responsible for fruity and fresh notes, were found in lower amounts in these jams. However, the overripe strawberry note found by the panelists could not be related to a significantly higher amount of any detected com-

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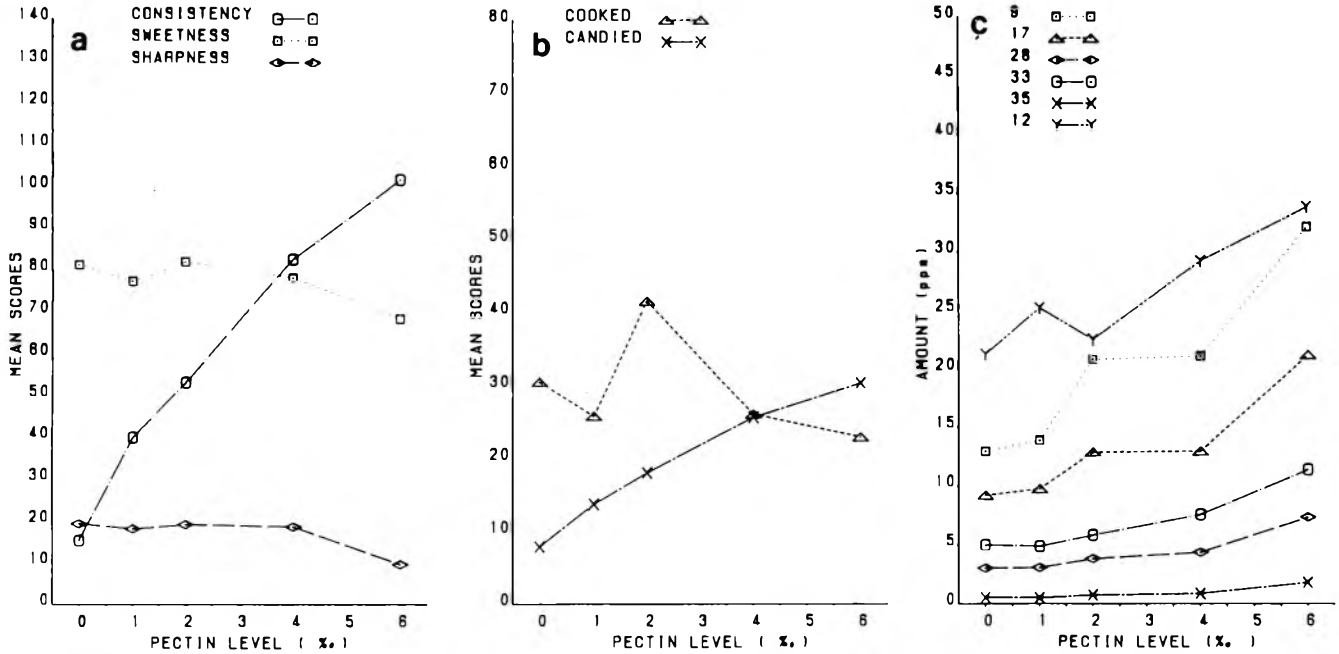

Fig. 3—Effect of LM pectin level: a,b: on sensory characteristics; c: on volatile composition in head-space. (Numbers refer to Table 2).

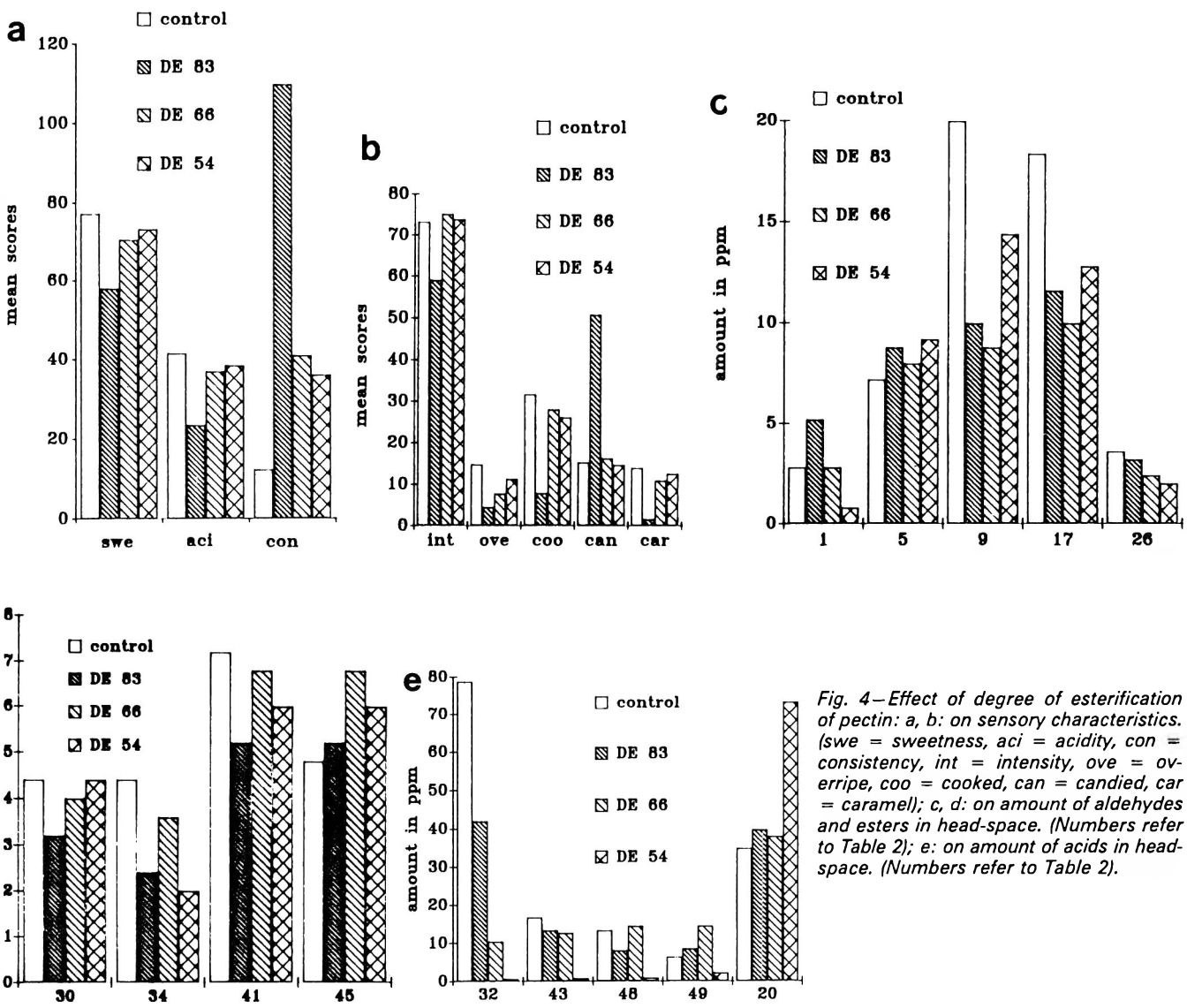

Fig. 4—Effect of degree of esterification of pectin: a, b: on sensory characteristics. (swe = sweetness, aci = acidity, con = consistency, int = intensity, ove = overripe, coo = cooked, can = candied, car = caramel); c, d: on amount of aldehydes and esters in head-space. (Numbers refer to Table 2); e: on amount of acids in head-space. (Numbers refer to Table 2).

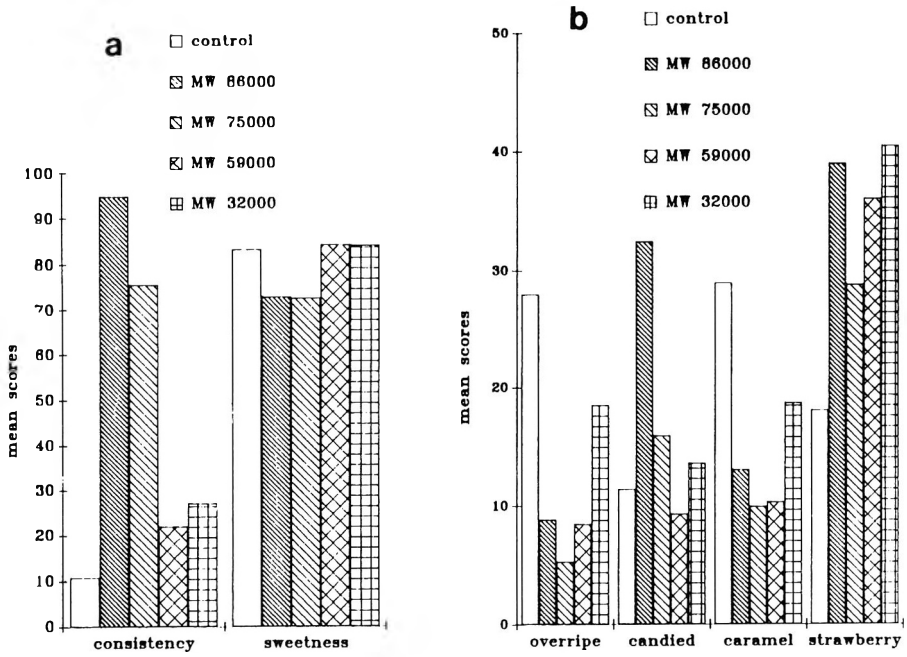

Fig. 5—Effect of molecular weight of pectin: a, b: on sensory characteristics.

pounds. Moreover, the addition of pectin, whatever the molecular weight, seemed to reduce the perception of the overripe note and to increase perception of the strawberry note. However, no significant difference was observed between the jams, in the head-space analysis. It was thus impossible, in this last experiment, to explain sensory assessment of the jam with analysis of head-space volatiles.

DISCUSSION

AT THE SAME CONCENTRATION, HMP gave a harder gel than LMP. This was confirmed by Unipectine Co. which advised use of HMP at a level of 0.2% and the LMP at a level of 0.4 to 0.6% to obtain similar gel strengths in 60°Brix jam. Moreover, commercial LM pectin was, in our case, a more efficient gelling agent than the two deesterified pectins. With its low degree of esterification, that pectin was more sensitive to calcium ions than the demethylated pectins, giving a more structured gel.

Our results showed that addition of pectin not only modified the oral consistency of jam, but could also cause a decrease in both taste and flavor intensities, thus confirming previous studied. For a specified pectin, this masking effect increased with level of pectin and, at a specified level, depended on the type of pectin. Since the gelling capacity changed with the type of pectin, this masking effect could be due only to an increase in consistency. As emphasized by Kokini et al. (1982) "to be tasted, a substance must diffuse to the surface of the taste buds and when the rate of diffusion is slower than the rate of the tasting reaction, the reaction is controlled by the diffusion rate". Thus, the taste intensity decreased with increasing consistency as the diffusion rate decreased. However, at a similar level of consistency, taste intensity was reduced less with a LMP than with a HMP, demonstrating that consistency was not the only factor responsible for variations in taste intensity.

The same pattern was observed for flavor intensity. Increases in the candied fruit note with increasing consistency could be due to the observed decrease of the amount of some volatiles responsible for the fresh strawberry note in the head-space. Subjects could have used this term to describe not only a specific flavor characteristic but a joint effect of a harder consistency and a lower fresh fruit intensity.

The amounts of many volatile compounds isolated from the different jams were not significantly different. However, due to the isolation procedure used, several important key components of jam aroma were not determined. This method did not allow, for example, the detection of polar compounds such as furaneol and lactones. Another experiment was done, to increase the amount of volatile compounds, in which the jelly was continuously and slowly mixed during the first 2 hr of stripping. The amount of collected volatiles, as expected, significantly increased, but there was no more significant difference between the jam prepared without added pectin and the jam prepared with 0.4% HMP. This suggested that the decrease in aroma intensity observed was due to very weak interactions between pectin and the volatile compounds. This was probably due to a slower diffusion, due to trapping of the small molecules between the pectin chains in the gel. Breaking the gel could lead to liberation of these molecules, thus reducing the pectin effect.

Demethylation of the pectin (experiment HDE), reduced the interactions between pectin and the volatile compounds, thus allowing a better perception of the aroma. However, demethylated pectins gave a less gellified jam, the consistency of which allowed more exchanges between the aqueous phase and the vapour phase. It is thus difficult, from this experiment, to dissociate the two effects and to conclude there was a direct effect of the degree of methylation.

CONCLUSION

AN IDEAL THICKENING AGENT should not interfere with the odor, flavor or taste of the product to which it is added (Pangborn et al., 1978). Our results show that addition of pectin can considerably change the flavor and taste of jam. A decrease in flavor intensity and in typical flavor was observed together with a decrease in fresh, overripe, cooked, and caramel notes, in sweetness, acidity and sharpness and a parallel increase in candied fruit note. This effect depended on the level and nature of pectin. LMP was more suitable than HMP for increasing consistency to the ideal level with no drastic changes in product flavor.

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differences for processing type and apple variety. Process juices were correctly allocated to their a priori process type and significantly different at $P<0.01$, except for 'diffuser juices' and 'enzyme liquefaction juices'. They were nearer on the plot ($P<0.05$) indicating their distinct analytical process differences (Fig. 1) were not reflected in the sensory evaluation. As with the analytical evaluation, the sensory evaluation showed varietal differences to be less distinct than process differences. The 'Spartan' juices were not different from either Delicious or Golden Delicious juices.

Effects of apple cultivar (Poll, 1981; Cliff and Dever, 1990) and apple storage (Dever and Cliff, 1990) have been shown to affect juice aroma, flavor and composition. Our study demonstrated clearly that the different extraction processes produced unique juices with significant analytical and sensory differences. When comparing effects of extraction process and apple cultivar, the extraction process had more influence on juice character.

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Low Dose Irradiation Influence on Yield and Quality of Fruit Juice

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ABSTRACT

Granny Smith apples, Valencia oranges, and Pearlette grapes grown in Queensland, Australia were irradiated at 0, 75, 300 and 600 Gy. Following irradiation, juice was extracted and analysed for quality and compositional changes. Irradiation treatment significantly ($p<0.05$) decreased yield of apple juice (by 6.3% w/w at 600 Gy) and grape juice (by 4.8% w/w at 600 Gy) but did not significantly ($p>0.05$) affect yield of orange juice (OJ). Acceptability significantly ($p<0.05$) decreased in OJ after 600 Gy treatment. Other changes in quality and composition were minimal. Juice from irradiated apples and oranges stored at 5°C for 8 wk showed similar results.

INTRODUCTION

ADVANTAGES claimed for food irradiation include the assertion that irradiation of fruit leads to increased juice yields (Anonymous 1982, 1988; Giddings 1983; Kader 1986; Loaharanu 1987). These assertions appear to be based on the work of Panhwer (1972) and Kiss et al. (1974). Panhwer (1972) reported radiation treatment at 4 and 8 kGy increased cell permeability, weakened skin and broke down protopectin and pectin between the cells of flesh, thereby increasing juice yields in different fruits and berries (cultivars not specified) by 7 to 10%. Kiss et al. (1974) reported that juice yield of five grape cultivars increased by 2 to 28% with increasing radiation dose of 0.5 to 16 kGy. This yield increase was attributed to increased permeability of cell walls. Wine fermented from grapes irradiated at doses higher than 8 kGy showed signs of deterioration whereas those from grapes treated with 0.5 kGy or 2 kGy were not impaired. Those doses were considerably higher than that required for disinfestation treatment of fruit, (75 Gy for control of Queensland fruit fly and 300 Gy to sterilize other insect species—International Consultative Group on Food Irradiation 1986), and are not likely to be employed in commercial practice. The objective of our study was to investigate the effect of low dose gamma irradiation on juice yield and quality.

MATERIALS & METHODS

Fruit

Valencia oranges (20 kg cartons) were purchased from the Brisbane markets in November, 1988. Pearlette table grapes (10 kg cartons) were harvested from the Granite Belt region of south east Queensland in January, 1989. Granny Smith apples (20 kg cartons) were purchased from the Brisbane markets in March, 1989. All commodities were held at 5°C until irradiated (within 5 days after purchase).

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Experimental design and statistical analysis

The experiments included replicated treatments of the three commodities. Effects of irradiation on apples and oranges were monitored at two stages: before storage (within 7 days after irradiation) and after storage at 5°C for 8 wk. Grapes were not stored and were examined within 7 days after irradiation. For all commodities, irradiation treatment consisted of four doses (0, 75, 300 and 600 Gy) with five replicates. Treatments were on 1 carton of apples, 1 carton of oranges or 2 cartons (20 kg total) of grapes. Another 6 cartons of each commodity were available for dosimetry. Data from replicated trials were analysed by analysis of variance (ANOVA). Where the ANOVA indicated a significant F value ($p < 0.05$), least significant differences were calculated for pairwise comparisons of treatment means. Unless otherwise stated, treatment means were compared on the same day and not across days (storage times).

Irradiation treatment

Dosimetry testing for the three commodities was on spare cartons of fruit on the day prior to irradiation. Absorbed dose was determined by spectrophotometric analysis of Fricke ferrous ammonium sulphate solution (Hart and Fricke, 1967) of dosimeters placed at several locations in representative cartons of fruit. Specified doses were to be minimum in all cases. Cartons of produce were stacked on each of four turntables in a small batch plant (GATRI) with a ^{60}Co plaque source (Izard, 1985) with an activity of 69 000 curies. Turntables were located 1 m from the source and were rotated during irradiation (about ¼ turn/15 sec). Fruit were held near 5°C at commencement of irradiation. Fruit were returned to the 5°C room shortly after irradiation.

Juice extraction

Granny Smith apples were weighed and washed up with tap water sprays on a continuous belt washer (Berry Wisher, Brisbane, Qld, Australia). Cores were manually removed with stainless steel apple corers and the fruit were cut into quarters with stainless steel knives. Pieces were pulped in a wet hammermill (J.A. Perkins and Co, Brisbane, Qld, Australia) fitted with 3 mm screen. Ascorbic acid (2 g/kg) was mixed with the juice to prevent darkening. The pulpy juice was finished by double passes through a Sardik^R (Sardik Engineering Co, Pyrmont, NSW, Australia) brush finisher fitted with screens of 1.5 mm diam holes (first-pass) and 0.8 mm holes (second-pass). Juice was weighed, filled into 25 L PVC drums with screw caps, and stored at 5°C. Valencia oranges were weighed, washed with tap water sprays on a continuous belt washer, and inspected for quality defects. Moldy fruit were removed. Fruit were manually halved with stainless steel knives and juice was manually extracted with a mechanical high speed stainles steel reamer. Juice was finished through a Sardik^R brush-finisher (Sardik Engineering Co) fitted with a medium size (1.5 mm diam holes) screen. Juice was then weighed, filled into 25 L PVC drums with screw caps and stored at 5°C. Pearlette grape bunches were weighed and then destemmed and crushed in an Amos^R destemmer-crusher (Amos Maschinenfabrik KG, Heilbronn, West Germany). Sodium metabisulphite solution (60 mg/kg SO_2) and dry ice pellets (solid CO_2) were added to the crush to prevent oxidation and darkening. Juice was extracted in a Willmes Press, (Josef Willmes, Bensheim/Hessen, West Germany) with two pressings at 100 kPa, and one at 200 kPa. Juice from all pressings was combined, weighed, filled into 25 L PVC drums with screw caps and stored at 5°C.

Juice analysis

Juice quality was measured by analysis of soluble solids (°Brix), pH, acidity, settling, viscosity, colour, pectinesterase activity, total

Table 1—Effect of irradiation on yield and quality of apple juice (juiced 6 days after irradiation).[a]

Parameter	Units	Dose (Gy) 0	75	300	600	S.E.
Juice yield	% w/w	54.9 a	56.0 a	50.0 b	48.6 b	1.351
°Brix		10.06 a	9.82 a	9.48 a	9.88 a	0.149
pH	–	3.29 a	3.24 b	3.14 c	3.09 d	0.005
Acidity	%	0.79 ab	0.81 a	0.82 a	0.77 b	0.011
Settling	mL	38.6 a	26.6 ab	14.2 b	29.2 ab	5.106
Viscosity	CP	65.6 a	266.4 a	113.2 a	221.2 a	92.497
Color L	–	56.5 a	56.3 ab	55.6 bc	55.0 c	0.297
Color a	–	−4.3 a	−5.0 b	−5.2 b	−5.2 b	0.081
Color b	–	19.4 a	19.1 a	19.2 a	19.1 a	0.113
Pectin esterase	PEU	0.11 a	0.13 a	0.18 b	0.25 c	0.009
Fructose	g/100 mL	4.21 a	3.82 a	3.93 a	3.80 a	0.251
Glucose	g/100 mL	1.39 a	1.22 a	1.30 a	1.20 a	0.078
Sucrose	g/100 mL	2.16 a	1.85 a	1.98 a	1.85 a	0.156
Citric	mg/mL	ND	ND	ND	ND	–
Malic	mg/mL	1.94 a	1.92 a	1.92 a	1.62 b	0.043
Tartaric	mg/mL	ND	ND	ND	ND	–
Acceptability[b]	–	3.9 a	4.6 a	4.7 a	4.1 a	0.247

[a] Means in a row followed by the same letter are not significantly different ($p = 0.05$). ND = not detected.
[b] By sensory test, 9 = like extremely, 1 = dislike extremely

vitamin C, dehydroascorbic acid, organic acids, sugars and taste panel assessment. Brix, pH and titratable acidity were determined by published methods (AOAC, 1984). For settling, juice was poured into a 250 mL measuring cylinder and allowed to stand 24 hr at ambient temperature. The volume of settled solids was recorded in mL. For viscosity, juice was poured into a 500 mL beaker and allowed to equilibrate at 20°C. Viscosity was measured with a Brookfield[R] viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA). Operating conditions were: spindle = LVF No. 3, angular velocity = 12 rpm, factor = 100. Viscosity was expressed in centipoise (CP) at 20°C.

Color was measured by Hunger L, a and b values, determined using a HunterLab[R] LabScan 6000 0°/45° spectrocolorimeter, IBM personal computer and software (Hunter Associates Laboratory, VA, USA). This instrument had a D65 illuminant and 10° observer and was standardised against a white tile (CIE 76.30, Y 81.03, Z 87.66). (L indicates lightness and darkness. Chromaticity coordinate a measures redness or greenness and b measures yellowness or blueness).

Pectin methylesterase activity was determined by a modification of the titrimetric procedure of Nagy et al. (1977). A 1% pectin-salt solution was freshly prepared with 75 mL 2 M NaCl made up to 1L with distilled water and blended with 10g Genu[R] pectin, Type LM 101 AS (The Copenhagen Pectin Factory Ltd, Denmark). The pectin-salt solution (110g) was placed in a 250 mL beaker held at 35°C in a forced circulation water bath. The juice sample (11g) was added and the mixture was blended with a Bamix[R] blender (Bamix, Melbourne, Vic, Australia) at high speed for 4 min. The mixture (100g) was weighed and placed in a covered flask in the water bath with overhead stirrer, pH electrode, and Metrohm[R] autotitrator (Metrohm, Herisau, Switzerland). The flask was continually flushed with nitrogen gas (1 L/min) and the mixture constantly stirred. NaOH (1N) was added to pH 6.5. Further NaOH (0.1N) was added to pH 7.1. The pH was allowed to decrease to 7.0 and the autotitrator and timer were started. NaOH (0.01 N) was automatically dispensed to maintain pH at 7.0 for 10 min, after which the titre was recorded. Enzyme activity was expressed in pectin esterase untis (P.E.U.) (Owusu-yaw et al., 1988):

$$PEU = \frac{(\text{mL NaOH} \times \text{normality of NaOH})}{(\text{g sample} \times \text{time (min)})} \times 10^3$$

Total vitamin C activity (ascorbic plus dehydroascorbic acid) was determined by a microfluorometric method (AOAC 1984) with exception that a Waters HPLC system (Waters Associates, Milford, MA) was used to transfer samples to a fluorescence detector. This system consisted of a model 510 pump, a WISP automatic sample injector, a model 420 fluorescence detector and a model 740 data module. Water was the transport medium at 1.0 mL/min. The sample (150 μL) was injected and vitamin C activity was detected at excitation wavelength 350 nm and emission wavelength 430 nm. Dehydroascorbic acid was determined in a similar manner to total vitamin C by omitting Norit (i.e. the oxidation step) in the extraction procedure.

For organic acids an aliquot of each juice (10 g) was centrifuged at 150 000 rpm for 20 min at a Sorvall Model RC5C centrifuge using an SS34 rotor (DuPont Company, Wilmington, Delaware, USA). Two mL of supernatant was filtered through Whatman GF/C glass fibre filter paper (Whatman Inc., Clifton, NJ) and diluted with 2 mL of distilled water. About 2 mL of filtrate was then filtered through a 0.45μ filter (Millipore HVLP 01300; Millipore Corporation, Bedford, MA) into sampler vials. Chromatographic analysis was on a Shimadzu HPLC system (Shimadzu Corporation, Kyoto, Japan) which comprised an SIL-6A automatic sample injector, two LC-6A pumps, a CTO column oven, an SCL-6A spectrophotometric detector and a CR4A integrator. An Aminex HPX-87H organic acid analysis column (Bio-Rad Laboratories P/L, Hornsby, NSW) was used, preceded by a Micro-Guard Cation-H guard column (Bio-Rad Laboratories). The column was operated at 65°C using 0.01N H_2SO_4 as mobile phase, at 0.6 mL/min. Five μL of sample was injected and eluting compounds were detected by absorbance at 210 nm. Quantification was by measurement of peak heights.

Extracts for sugar analysis were prepared by the same procedure as for organic acids. Chromatography was on a HPLC system which comprised a Waters Wisp automatic sample injector, a model 510 pump, a Bio-Rad column heater and an ERMA ERC-7510 refractive index detector coupled to a Shimadzu CR3A computing integrator. A Waters Sugar-PAK 1 column was used, preceded by a Waters Guard-PAK precolumn module. The column was operated at 70–90°C using calcium ethylene diamine tetraacetate (50 mg/L) at 0.5 mL/min as mobile phase. The sample (50 μL) was injected and eluting compounds were detected by differential refractive index and quantitated by peak area measurements.

Taste panel assessment

Samples of control and irradiated juice were presented to a 12-member experienced taste panel who were asked to rate each sample for general acceptability using a nine-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely).

RESULTS & DISCUSSION

THROUGHOUT THE STUDY, dosimetry testing indicated the average dose rate was 7.7 Gy/min with a maximum to minimum dose ratio of 1.3/1.

Juice extraction effects

The results for juice yield and quality factors are presented in Table 1 to 5. Juice yield showed a significant ($p<0.05$) decrease with increasing irradiation dose for stored and unstored apples and grapes. Orange juice yield was not affected by irradiation treatment. The increase in juice yield associated with irradiation treatment reported in previous studies (Panhwer 1972; Kiss et al., 1974) has been associated with deterioration in fruit texture. This has been attributed to one or more radiation-induced changes including: increased permeability of the walls of the skins, weakening of the skin, breakdown of the protopectin and pectin of intermediate layers which weakens the bonds between the cells. Especially the bonds between the skin and the parenchymatous cells and between

Table 2—Effect of irradiation on yield and quality of stored apple juice[a,b]

Parameter	Units	Dose (Gy) 0	75	300	600	S.E.
Juice yield	% w/w	53.2 a	49.1 b	46.5 b	47.4 b	1.222
°Brix		11.26 a	11.04 ab	10.86 b	10.90 b	0.092
pH	–	3.04 a	3.10 b	3.12 b	3.04 a	0.010
Acidity	%	0.68 a	0.65 ab	0.63 bc	0.60 c	0.012
Settling	mL	41.6 a	51.4 a	39.6 a	36.4 a	6.380
Viscosity	CP	50.0 a	48.6 a	74.0 a	148.4 b	14.035
Colour L	–	46.2 a	44.3 a	45.7 a	45.0 a	0.771
Colour a	–	−5.3 a	−3.3 b	−5.3 a	−3.9 b	0.303
Colour b	–	19.7 a	18.1 a	17.3 a	17.8 a	1.154
Pectin esterase	PEU	0.32 a	0.22 b	0.28 c	0.26 c	0.014
Fructose	g/100 mL	4.81 a	5.50 a	4.65 a	5.49 a	0.243
Glucose	g/100 mL	1.79 a	2.00 a	1.78 a	2.01 a	0.090
Sucrose	g/100 mL	2.95 a	3.38 a	2.72 a	3.25 a	0.169
Citric	mg/mL	ND	ND	ND	ND	–
Malic	mg/mL	2.13 a	2.13 a	2.03 a	1.79 a	.126
Tartaric	mg/mL	ND	ND	ND	ND	–

[a] Stored at 5°C for 8 wks after irradiation.
[b] Means in a row followed by the same letter are not significantly different (p = 0.05).
ND = not detected.

Table 3—Effect of irradiation on yield and quality of orange juice (juiced 7 days after irradiation)

Parameter	Units	Dose (Gy) 0	75	300	600	S.E.
Juice yield	% w/w	58.7 a	59.3 a	59.0 a	60.1 a	0.323
°Brix		10.1 a	10.4 a	10.4 a	10.4 a	0.210
pH	–	3.56 a	3.54 a	3.57 a	3.59 a	0.033
Acidity	%	0.83 a	0.89 a	0.91 a	0.86 a	0.050
Settling	mL	59.6 a	62.0 a	60.8 a	64.8 a	2.736
Viscosity	CP	60.0 a	60.2 a	57.2 a	69.4 a	3.906
Colour L	–	34.5 a	37.8 a	36.9 a	41.4 a	1.625
Colour a	–	7.8 a	9.7 a	9.3 a	12.2 a	1.053
Colour b	–	19.2 a	21.5 a	20.7 a	23.9 a	1.217
Pectin esterase	PEU	3.11 a	3.52 b	3.15 a	3.21 a	0.099
Total Vitamin C	mg/100 mL	40.40 a	41.60 a	41.62 a	41.34 a	0.839
Dehydroascorbic	mg/100 mL	7.90 a	6.08 a	6.48 a	11.44 b	0.852
Fructose	g/100 mL	1.35 a	1.40 a	1.40 a	1.41 a	0.102
Glucose	g/100 mL	1.21 a	1.24 a	1.25 a	1.25 a	0.095
Sucrose	g/100 mL	2.67 a	2.60 a	2.58 a	2.63 a	0.230
Citric	mg/mL	9.58 a	10.00 a	9.85 a	10.17 a	0.960
Malic	mg/mL	3.58 a	3.08 a	2.77 a	3.26 a	0.270
Tartaric	mg/mL	ND	ND	ND	ND	–
Acceptability[b]	–	6.9 a	6.8 a	6.6 a	6.1 b	0.141

[a] Means in a row followed by the same letter are not significantly different (p = 0.05).
ND = not detected.
[b] By sensory test, 9 = like extremely, 1 = dislike extremely

Table 4—Effect of irradiation on yield and quality of stored orange juice (stored at 5°C for 8 wk after irradiation).[a]

Parameter	Units	Dose (Gy) 0	75	300	600	S.E.
Juice yield	% w/w	59.0 a	58.5 a	58.9 a	59.2 a	0.689
°Brix		10.2 a	10.7 a	10.6 a	9.8 a	0.270
pH	–	3.74 ac	3.63 b	3.68 bc	3.79 a	0.031
Acidity	%	0.76 a	0.91 a	0.86 a	0.73 a	0.060
Settling	mL	51.8 a	48.8 a	46.0 a	43.2 a	2.325
Viscosity	CP	32.0 a	27.6 a	37.4 a	33.2 a	2.979
Colour L	–	49.5 a	50.5 a	48.7 a	50.2 a	0.437
Colour a	–	10.5 ab	11.2 b	9.8 a	10.8 b	0.277
Colour b	–	56.9 a	57.3 a	54.5 b	56.6 a	0.656
Pectin esterase	PEU	2.12 a	2.53 b	2.52 b	2.98 c	0.124
Total Vitamin C	mg/100 mL	42.30 a	41.16 a	40.76 a	39.52 a	1.277
Dehydroascorbic	mg/100 mL	3.62 a	5.34 a	5.24 a	6.16 a	0.903
Fructose	g/100 mL	1.67 a	1.54 a	1.48 a	1.02 a	0.186
Glucose	g/100 mL	1.52 a	1.40 a	1.34 a	0.91 a	0.173
Sucrose	g/100 mL	3.40 a	2.94 a	2.76 a	1.95 a	0.366
Citric	mg/mL	9.86 a	11.00 a	11.39 a	9.53 a	0.714
Malic	mg/mL	4.00 a	3.67 ab	3.45 b	3.51 b	0.123
Tartaric	mg/mL	ND	ND	ND	ND	–
Acceptability[b]	–	6.4 a	6.7 a	6.3 a	5.0 a	0.441

[a] Means in a row followed by the same letter are not significantly different (p = 0.05).
ND = not detected.
[b] By sensory test, 9 = like extremely, 1 = dislike extremely

the cells of the flesh have been reported weakened (Ahmad and Hassain, 1973; Urbain, 1986).

Table 5—Effect of irradiation on yield and quality of grape juice (juiced 7 days after irradiation)

Parameter	Units	Dose (Gy) 0	75	300	600	S.E.
Juice yield	% w/w	50.3 a	50.3 a	49.5 a	45.5 b	0.822
°Brix		16.34 a	16.44 a	16.74 a	16.56 a	0.411
pH	–	3.70 a	3.74 a	3.71 a	3.72 a	0.022
Acidity	%	0.56 a	0.55 a	0.55 a	0.57 a	0.012
Settling	mL	89.2 a	89.4 a	91.4 a	89.4 a	1.771
Viscosity	CP	40.0 a	40.4 a	36.6 a	34.8 a	3.985
Colour L	–	34.2 a	35.1 ab	35.5 b	37.1 c	0.417
Colour a	–	−1.4 a	−1.4 a	−1.7 b	−2.0 c	0.071
Colour b	–	4.9 a	5.3 ab	5.6 bc	5.8 c	0.111
Pectin esterase	PEU	0.05 a	0.04 a	0.04 a	0.05 a	0.004
Total Vitamin C	mg/100 mL	40.0 a	40.4 a	36.6 a	34.8 a	3.985
Dehydroascorbic	mg/100 mL	6.3 a	6.4 a	6.7 a	6.6 a	0.411
Fructose	g/100 mL	6.43 a	9.22 a	7.93 a	7.50 a	0.646
Glucose	g/100 mL	5.94 a	8.58 a	7.32 a	6.92 a	0.584
Sucrose	g/100 mL	ND	ND	ND	ND	–
Citric	mg/mL	0.18 a	0.19 a	0.19 a	0.21 a	0.009
Malic	mg/mL	3.79 a	3.67 a	3.68 a	3.92 a	0.309
Tartaric	mg/mL	4.50 a	5.09 a	5.22 a	5.13 a	0.484
Acceptability[b]	–	6.6 a	6.3 a	6.4 a	6.5 a	0.115

[a] Means in a row followed by the same letter are not significantly different (p = 0.05).
ND = not detected.
[b] By sensory test, 9 = like extremely, 1 = dislike extremely.

The extent of these changes would depend on irradiation dose employed. In our study apparently the doses employed were not sufficient to cause such changes and hence cause increased juice yields. The yields recorded for the three commodities, (45.5 to 60.1%) were lower than expected from normal juice extraction (Tressler and Joslyn 1971). This could be attributed to the pilot-scale nature of the extraction procedure. As expected, yields of juice for stored fruit were lower than those for unstored fruit (Tressler and Joslyn 1971). Table 5 shows 5% lower juice yield for 600 Gy treated grapes than for non-irradiated grapes. Kiss et al (1974) recorded yield increases of 0 to 7.1% for five varieties of wine grapes irradiated at 500 Gy. The conflicting results may have been due to varietal differences.

Effects on juice quality

For unstored applies (Table 1), increasing irradiation dose produced significant ($p<0.05$) decreases in pH, settling, Hunter 'L' value (brightness) and 'a' value (greeness). In stored apples, increasing irradiation dose resulted in a significant ($p<0.05$) decrease in °Brix, acidity and pectinesterase activity (Table 2). Juice from 600 Gy treated, stored apples was significantly more viscous than that from all other treatments. Taste panel scores from apple juice were low, with panelists indicating a metallic, processed type flavour. This was probably due to the

method of preparation of the juice extract. Eric et al. (1970) recorded flavor and color differences in Granny Smith apples following irradiation at 250, 500 and 1000 Gy. They also reported no significant ($p > 0.05$) changes in vitamin C due to irradiation at 205 or 500 Gy.

Changes occurring in juice from unstored and stored oranges were minimal. Sensory assessment of juice from 600 Gy treated unstored oranges showed it was significantly ($p < 0.05$) less acceptable than juice from any other treatments (Table 3). The 600 Gy treated juice also had a significant ($p < 0.05$) increase in dehydroascorbic acid. In stored oranges, increasing irradiation dose was associated with significant ($p < 0.05$) increase in pectinesterase activity and decrease in malic acid (Table 4). MacFarlane and Roberts (1968) reported only minimal changes for flavor, Brix, acidity and vitamin C in Valencia oranges irradiated at 250 to 1000 Gy. Nagai and Moy (1985) reported Valencia oranges treated with 750 and 1000 Gy were significantly ($p < 0.05$) lower in flavor scores than controls or fruit irradiated at 300 or 500 Gy.

For grape juice, the only significant ($p < 0.05$) changes recorded were for colour, with an increase in Hunter 'L' (brightness) and 'b' (yellowness) values and a decrease in 'a' value (greeness) associated with increasing irradiation dose (Table 5). Irradiation treatment resulted in a significant ($p < 0.05$) decrease in pectinesterase activity for stored apples and a significant ($p < 0.05$) increase for stored oranges. Pectinesterase activity increases clarification of fruit juice. This results when pectinesterase demthylates pectin which in turn brings about formation of calcium pectate and subsequent colloidal and physical changes causing clarification (Tressler and Joslyn 1973). Irradiation of fruit previously has been shown to cause increases in water soluble pectin and hence increases in juice viscosity (Dennison and Ahmed 1966; Josephson and Frankfort 1967). This was found in all samples.

CONCLUSIONS

LOW DOSE IRRADIATION (< 600 Gy) did not appear to result in increased juice yield. Irradiation treatment resulted in significant decreases in juice yields from apples and grapes and no change in juice yield from oranges. Irradiation treatment caused only nominal changes in fruit juice composition.

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Orange Juices and Concentrates Stabilization by a Proteic Inhibitor of Pectin Methylesterase

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ABSTRACT

A proteic inhibitor of pectin methylesterase (PME), recently discovered in kiwi, was used to stabilize cloud of orange juice concentrate with "Cut-back," 42° Brix. To concentrated and pasteurized orange juice were added increasing amounts of fresh juice (12° Brix) with PME 0.8 U/mL at the fresh to pasteurized ratios 6.6 to 38%. The PME inhibitor (65% pure), from kiwi, was added at 50 mg/L. After 8 mo at 5°C samples were compared with controls containing the same amount of fresh juice with PME inhibited by pasteurization. Pasteurized samples were not different from those treated with PME inhibitor. Conversely, where PME was not inhibited, cloud stability decreased with increasing amounts of fresh juice. Use of kiwi PME inhibitor in fruit juice production has potential advantages.

INTRODUCTION

FRUIT JUICES and concentrates are biphasic systems made up of a liquid phase, generally termed "serum" and a solid phase which, in orange juice, is termed "cloud." A main objective of citrus juice technology is preservation of physical characteristics of cloud. Soluble pectin is that part of juice that stabilizes cloud. When this colloid is demethylated by pectin methylesterase (PME), naturally present in juice, it causes clarification of the juice because of rapid sedimentation of the solid phase. This is due to formation of free carboxyl groups by PME action on pectin. These carboxyl groups react with calcium ions present in the juice (Primo Yufera et al., 1961; Baker et al., 1969). Since the resulting calcium pectate is insoluble, it precipitates, destabilizing cloud with consequent clarification (Stevens et al., 1950; Krop and Pilnik, 1974). Several technological approaches have been used to solve the problem of juice clarification. The yare summarized as follows: Thermal treatment (105–115°C) to inactivate PME. This is the most common technology used for cloud stabilization. However, care must be taken to avoid change in juice flavor from the thermal process (Cruess, 1914; Irish, 1928). Inhibition of PME by storing juices at low temperature. This is usually employed for "Frozen" products, obtained by mixing concentrated juice with fresh unpasteurized juice. Degradation of PME substrate. This is not frequently used. It consists of degrading soluble pectin to a low degree of methylation, with enzymes such as pectin lyase or polygalacturonase that prevent pectin precipitation by calcium through reduction of pectin molecular weight (Baker and Bruemmer, 1969; 1972; Baker, 1977). Inactivation of PME by proteolytic enzymes. Recently Wobben and Tan (1983) patented a process to stabilize cloud in citrus beverage by subjecting pasteurized concentrate, or single strength juice, to one or more enzymes with protease activity. They claimed cloud stability depended on the degree of protein hydrolysis in the juice.

Our objective was to explore a novel way to preserve cloud stability. We applied our recent finding that kiwi fruit contains a powerful proteic inhibitor (Balestrieri et al., 1990). This substance, which we demonstrated to be a glycoprotein, was partially purified and used as an additive to "cut-back" juice in juice concentrates in order to stabilize cloud through PME inhibition.

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MATERIALS & METHODS

Reagents

Citrus pectin and orange PME were purchased from Sigma Chemical Co. (St. Louis, MO). Q-sepharose was obtained from Pharmacia (Uppsala, Sweden). All other reagents were analytical grade.

Determination of pectin and PME activity

PME activity was determined titrimetrically by the method of Vas et al. (1967) estimating free carboxyl groups formed in pectin as a result of enzyme action. The amount of 0.01M NaOH required to maintain the substrate at pH 7.0 (30°C), was measured using an automatic titrator. The enzyme substrate was a 1% citrus pectin solution containing 0.15M NaCl. One unit of PME was defined as the amount of enzyme which released 1 μmole of carboxyl groups/minute. The pectin content in orange juice was determined according to the method of Rouse and Atkins (1964).

Purification of PME inhibitor from kiwi fruit

Pectin methylesterase inhibitor (PMEI) was partially purified from kiwi fruit essentially as described by Balestrieri et al. (1990). Briefly, 15 kg of kiwi were washed and homogenized in water, the suspension was then passed through a depulper with a 0.4 mm filter. The filtrate was centrifuged and the supernatant adjusted to pH 7.5 by adding NaOH. After dialysis against Tris 10 mM, pH 7.5, 100 mM NaCl, the solution was mixed with Q-Sepharose resin equilibrated in the same buffer. After mixing 3 hr at 4°C, the slurry was poured onto a column (5 × 70 cm) and the resin was allowed to settle. The column was washed with buffer containing 10 mM Tris-HCl pH 7.5, 100 mM NaCl, and PMEI was eluted with a linear gradient for 100 to 400 mM NaCl. Fractions containing PMEI activity were pooled and concentrated by ultrafiltration. The concentrated solution, free of polygalacturonase and proteinase activity, was used as a source of PMEI. From 15 kg of fruit, 600 mg of proteins were obtained, and 65% was PMEI.

Samples preparation

To evaluate PMEI ability to stabilize orange juices, samples were prepared from pasteurized concentrated orange juices (COJ) at 62°Brix. Fresh unpasteurized orange juices at 12°Brix, previously depulped by filtration, were than added to the pasteurized concentrates. The PME activity in the depulped juice was 0.84 U/mL, whereas no activity was found in the concentrated/pasteurized juice. The final concentration in the "cut-back" concentrates was 42°Brix and the amount of the added unpasteurized orange juice ranged from 6.6 to 38%. Figure 1 outlines the preparation scheme for samples. At the indicated concentration of fresh juice, each sample was separated into three lots and treated as follows: Samples labeled P: pasteurized at 100°C for 7 min. Samples labeled NP: unpasteurized. Samples labeled I: containing enough PMEI (50 mg/L) to completely inhibit PME in the juice. Each batch was treated with 2000 ppm benzoic acid and 1000 ppm sulfur dioxide and divided into 20 samples, stored in sealed tubes at 5°C.

Cloud index determination

Concentrated juices at 42° Brix (20g) were diluted with water to 12° Brix. After centrifugation at 2000 × g for 10 min, supernatant transmittance at 660 nm was determined (Loeffler, 1941).

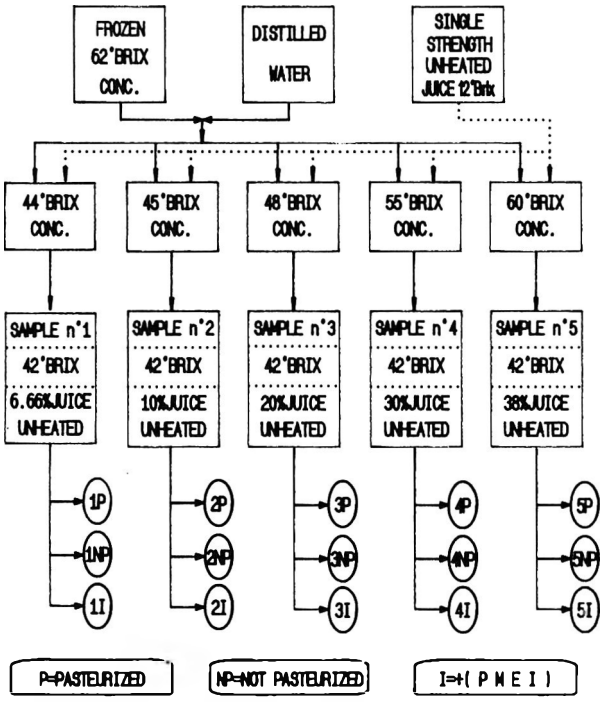

Fig. 1—Scheme of sample preparation for cloud stability test.

RESULTS & DISCUSSION

THE DISCOVERY and purification of a proteic inhibitor in kiwi fruit suggested to us that it may have potential in biotechnology processes, such as those regarding orange juices, for inhibition of PME activity. The effects of PMEI addition to concentrates with "Cut-back" juices are reported in Table 1. No PME activity was detectable in either pasteurized or in PMEI added juices at the beginning or at the end of the observation period. On the other hand, in samples prepared by mixing pasteurized and fresh juices, the relatively high PME activity at the start of the period decreased to zero during the 8 mo storage at 5°C (Table 1). This effect, of a gradual loss of PME activity with time in juice, has been reported by Koen Mosse et al. (1960). Moreover, they also observed a faster decrease of PME activity in stored juices with increased storage temperature.

The effect of PMEI addition on pectic fractions in various "cut-back" samples is reported in Table 2. Note that "cut-back" juices containing fresh juice with added PMEI, had the same composition of pectic fractions as the pasteurized samples. Conversely, in "cut-back" concentrates containing fresh juice without PMEI addition, the pectic fraction composition dramatically changed. They had an increase of sodium hexametaphosphate soluble pectins (calcium pectates) and a noticeable decrease in the water soluble fraction, whereas the NaOH soluble fraction (protopectin) remained unchanged. The same result was obtained when pH of the various samples was measured. We found no pH difference between juices with added PMEI and pasteurized juices. In contrast, the pH of samples with fresh juices, without PMEI, was notably lower (in some samples as much as 0.2 units). These results reemphasized the PMEI inhibitory effectiveness on PME activity even for long time periods.

Many reports have been published (Crandall et al., 1983; Wenzel et al., 1951; Rothschild et al., 1975) concerning factors affecting cloud stability of citrus juices and concentrates. These essentially covered effects of on fruit variety, seed content, pulp quality and quantity, concentration degree, storage temperature and residual PME activity. On the other hand, the clarification process in a juice concentrate is known to depend on total soluble solids, with other factors constant (i.e. pulp content, residual PME activity, storage temperature). Particularly, Cotton et al. (1947) observed that higher gelification and clarification occured when total soluble solids corresponded to 30–40° Brix, whereas juices with lower or higher soluble solids appeared more stable. Therefore, on the basis of those findings, PMEI was assayed under conditions of lowest juice stability, i.e., on those around 40° Brix or higher with increasing contents of PME activity, obtained by increasing the added fresh juice. Cloud stability curves for various "cut-back" juices prepared in this manner are reported in Fig. 2. Only samples with fresh juice added at a concentration up to 6,6%(PME activity about 0,9 U/mL) were stable throughout the observation period. When percent of fresh juice in the "cut-back" concentrate was above that value, decreased stability of the juice was found. However, addition of PMEI to samples with the same fresh juice content completely preserved cloud stability. Those juices appeared the same as corresponding pasteurized juices throughout the observation period. Technological use of the inhibitor is not limited to orange juice. Recently, we demonstrated that the inhibitor was equally active in blocking PME activity in other fruit juices (Castaldo et al., 1990).

Table 1—PME residual activity in concentrates containing "Cut-back" juices (42° Brix) stored at 5°C

PME activity (U/mL × 10^2)		
	Storage (day)	
Sample[a]	0	240
1P	0	0
1NP	5	0
1I	0	0
2P	0	0
2NP	8	trace
2I	0	0
3P	0	0
3NP	17	trace
3I	0	0
4P	0	0
4NP	34	trace
4I	0	0
5P	0	0
5NP	43	.trace
5I	0	0

[a] P = Pasteurized, NP = Not pasteurized, I = Containing pectinmethylesterase inhibitor.

Table 2—Pectic fraction composition of concentrates with "cut-back" juices after 240 days at 5°C

Pectin content (mg/kg juice)			
	Soluble in		
Sample[a]	Water	Sodium hexametaphosphate	NaOH
1P	1030	210	320
1NP	1020	220	340
1I	996	230	340
2P	1097	198	298
2NP	620	686	310
2I	1010	286	287
3P	1066	257	386
3NP	543	734	389
3I	937	411	428
4P	1000	270	407
4NP	298	860	447
4I	972	326	435
5P	1008	412	448
5NP	223	1052	407
5I	978	462	417

[a] P = Pasteurized, NP = Not pasteurized, I = Containing pectinmethylesterase inhibitor.

CONCLUSIONS

THE KIWI PMEI could be successfully used to control PME activity in orange juice even over long storage. This procedure

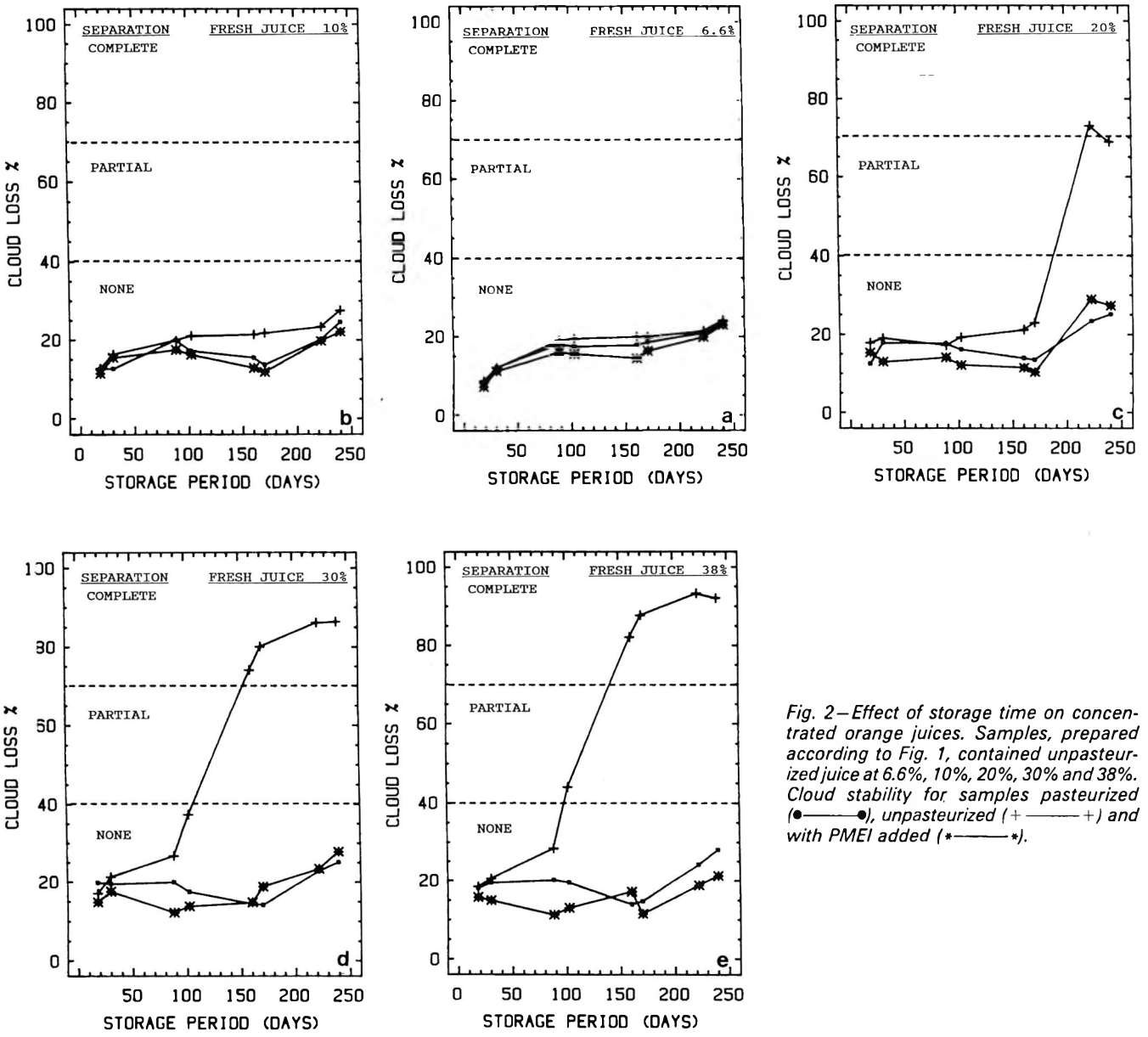

Fig. 2—Effect of storage time on concentrated orange juices. Samples, prepared according to Fig. 1, contained unpasteurized juice at 6.6%, 10%, 20%, 30% and 38%. Cloud stability for samples pasteurized (●———●), unpasteurized (+———+) and with PMEI added (———*).*

of PME inactivation could allow lower thermal juice treatment inasmuch as thermal PME inactivation requires temperatures higher than those needed for pasteurization. Such milder thermal treatment should result in better flavor quality. Moreover, the inhibitor could also be usefully employed in frozen product technology. After long-term storage at 5°C, our results showed that, in the presence of PMEI, the juice did not undergo changes characteristic of PME action. Thus, addition of PMEI might allow the storage of frozen products at higher temperatures.

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Filter, Fourier Transform Infrared, and Areometry, for Following Alcoholic Fermentation in Wines

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ABSTRACT

Areometry, filter near infrared spectrometry (FIR), and Fourier transform near infrared spectrometry (FTIR), have been compared for following total sugar and ethanol during fermentations. Standard errors of calibration (SEC) and prediction (SEP) have been estimated, respectively, with 4 and 2 fermentations, using as reference sugar and ethanol enzymatic analysis. With the first 4 fermentations, the least rectangles method was applied for a more thorough comparison between FIR, FTIR, and areometry. FIR was more complex and more accurate than density measurement, and less complex, but as accurate as FTIR spectrometry. Small SEP can be achieved with FIR spectrometry: 2.5 g/L for total sugars and 0.15% (v/v) for ethanol.

INTRODUCTION

WINE is made by transforming sugars into alcohol during fermentation of grape musts. In all wine fermentation processes, the winemaker must decide to refrigerate the must to slow down the fermentation process when controlling quality of the wine.

The decision to refrigerate the fermenting must takes into account the fermentation evolution rate. This is described as piloting the fermentation by means of temperature control (Sablayrolles et al., 1987). However, traditional practice has been an art because of the lack of adequate sensors. Temperature probes and manual density measurements (i.e. specific gravity) several times per day have been the only data available from which the stage of the fermentation and the evolution rate could be estimated. Continuous on-line automatic indications of fermentation stage and evolution rate would be great improvements in wine fermentation technology. Automating the traditional density measurements needs further research to evaluate its benefits such as optimal cost, reliability, and precision. In fact, any physical measurement which would enable estimation of the sugar or alcohol concentrations during fermentation might be a more applicable sensor. Measurement of CO_2 production has been recently implemented (El Haloui et al., 1987), but winemakers object to the required closing of the tanks.

Refractometry is less precise than density because accuracy of laboratory instruments in sugar determination is less. In addition, in a fermenting must, alcohol production counteracts partly the action of sugar consumption on the changes in refractometry index, while alcohol production reenforces the action of sugar consumption on the changes in density. These facts affect the range of variation in refractive index, making it relatively less than the density range during fermentation. Therefore, density is more adequate for estimating fermentation and evolution rate. From El Haloui et al. (1987), following the density of fermenting must permits estimation of sugar concentration evolution with a standard deviation of 8 g/L.

Low resolution NMR was used by Tellier et al. (1989) to determine residual sugar and alcohol in the course of fermentation. Their observed linear correlations between NMR signal and total sugar content at 0–200 g/L, and between NMR signal and ethanol content at 0–12 % (v/v), can be used to predict composition of the fermenting medium (standard deviations were 4–8 g/L for total sugar and 0.05 to 0.1 % (v/v) for ethanol). The calibration curves can be used to follow the kinetics of enological fermentation. The rapidity of the measurement (3.5 min) and the easy sample preparation make this technique suitable for on-line control of fermentation. However, the methodology needs to be improved to be competitive.

NIR spectrometry instruments have been applied to sugar content measurement in dry mixtures (Giangiacomo et al., 1981), in aqueous mixtures (Giangiacomo and Dull, 1986; Dumoulin et al., 1987), in fruit (Davenel et al., 1988), and in intact fruit (Lanza and Li, 1984). It has also been applied to estimate sugar content in wines (Kaffka and Jeskenszki, 1984; Cabanis et al., 1983). Fermenting musts have been followed by means of a Technicon 400 analyser (Bouvier, 1988), and standard deviations were less than 5 g/l for total sugar and less than 1% (v/v) for ethanol. The work of Giangiacomo, Davenel, Lanza, and Kaffka was done with grating instruments, while that of Dumoulin, Cabanis, and Bouvier used filter instruments. Grating instruments produce complete spectra and permit selection of wavelengths of interest for further work with filter instruments.

More recently, NIR spectometry using Fourier transform method (FTIR) has been used for on line measurements in food processing (Davenel et al., 1988; Davenel, 1990). As low resolution FTIR spectrometers (slight displacements of movable mirror) become available at lower cost, their consideration is worthwhile for following wine fermentation progress but no such attempt has been reported.

We decided to focus our study on density, FTIR, and FIR measurements for two reasons. They are among the most accurate physical methods for sugar and ethanol estimation. They have major differences, areometry being simple, traditional, and cheap, and near infrared spectrometry being complex and costly. For FIR, filter instruments were preferred over grating instruments because they are better adapted to industrial conditions.

MATERIALS & METHODS

THE FERMENTING TANK was a 150L stainless steel cylinder, coated for reproducing the thermal inertia of a 5000L industrial tank of same height. The tank was filled to 130 L. No mechanical mixing was used, as the tank content was homogenous because of fermentation activity.

Instrumentation

Four areometers, specific gravity or density manual measurement tools based on buoyancy, Dujardin-Salleyron (Paris, France), covering the range of the must density evolution during fermentation (1.090–1.060, 1.060–1.030, 1.030–1.000, 1.000–0.970) were used for manual density measurements of uptakes four times per day (precision 0.0001).

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Table 1—Main physical and chemical characteristics of musts before fermentation

Fermentation	F1	F2	F3	F4
Temperature (°C)	16.8	31.0	24.5	19.6
Specific gravity (g/L)	1088.2	1077.0	1089.6	1091.6
Refractive index	1.3655	1.3600	1.3670	1.3674
Ethanol (vol %)	0	0	0	0.5
Sugar (g/L)	200.4	163.4	227.6	228.3
Glycerol (g/L)	4	0	0.9	3.2

Two NIR spectrometry techniques were tested: a 400 Infralyser (Technicon Co, Dumont, France) equipped with 19 filters (popular in the food industry), and a Fourier transform interferometric spectrometer, the Nicolet 7199 (Nicolet Co, USA). Samples were analysed with the Infralyser at 22°C. The main operating parameters of the Nicolet 7199 spectrometer, equipped especially for NIR (separator CaF_2, detector InAs cooled), were from a previous theoretical study (Davenel, 1989): maximum optical path length difference of 0.0315 cm, no apodization, 100 scans per spectrum, 1 mm thick quartz cell. Absorbance data were obtained relative to a background scan for water. Measurement cells were kept at 22°C within ± 0.5°C. Each sample was scanned 100 times in 1 min and interferograms were averaged to give a simple transmittance spectrum after Fourier transformation. NIR data were processed to log(1/T), because no significant background variations arose, and because the second derivative method deteriorated the signal to noise ratio.

Fermentations

A calibration study was carried out with 4 fermentations involving Carignan cultivar and conducted so that temperatures were not allowed to exceed 30°C, except for the fermentation F2. The main characteristics of initial musts are presented in Table 1. Initial musts were reconstituted from concentrated juices (80 % volume reduction). Thirteen g/hL of *Saccharomyces cerevisiae*, strain K1 (INRA, Montpellier, France), inoculated 130L prepared must. To avoid foaming, 10 g/hL of Silicone 426 (Prolabo Co., Paris, France) were added. Some glycerol was in all musts before the experiments, except F2, and in addition the F4 must contained a significant amount of ethanol. This indicated the start of fermentation during storage of the concentrated juices in all cases but F2 (especially F4). The prediction study used 2 extra fermentations, with Macabeu and Carignan cultivars, which were conducted similarly to fermentation F1. Concentrated musts further diluted with water (making grape juice) were used, because they provided yearound experimentation and required smaller storage volumes. Although this experimental procedure should be extended to fresh grape juices, it provided a good way of investigating fermentation kinetics and sensors.

There were, on average, 4 uptakes of 0.5L must per day. Density was measured 2 min after uptaking, allowing foam to disappear. A non sterile filtration of 100 mL of must clarified it and it was sterilized with 2 drops of allylisothiocyanate (Merck Co, Hohenbrunn bei München, Germany). For each sample 3 subsamples were kept at 4°C for enzymatic analyses and further NIR measurements. Eighty-seven uptakes were taken for calibration as follows (for fermentations F1, 24, F2, 13, F3, 25, and F4, 25), and 30 for prediction (15 and 15).

Reference enzymatic analyses

The enzymatic analyses of ethanol, glucose, fructose and glycerol were automated (60 samples/hr) according to the techniques implemented by Battle and Bouvier (1986), with a precision of 3 g/L sugar, 0.1 g/L glycerol, and 0.1 % (v/v) ethanol.

Statistical methods for calibration and comparison

Each physical method compared to the reference enzymatic analysis was calibrated with linear least squares regressions. The selection of the spectrometer wavelengths was made using a forward stepwise regression (Osborne et al., 1986). Calibrations of each sensor had correlation coefficients above 98% for the instrumental response and the reference values. Thus the fit was estimated by the Standard Error of Calibration (SEC) (Osborne et al., 1986). Accuracies between methods were compared using the least rectangles method as recommended by Feinberg (1984), who referred to Danielli (1973), since this technique is perfectly symmetrical about both variables, as opposed to the least squares method.

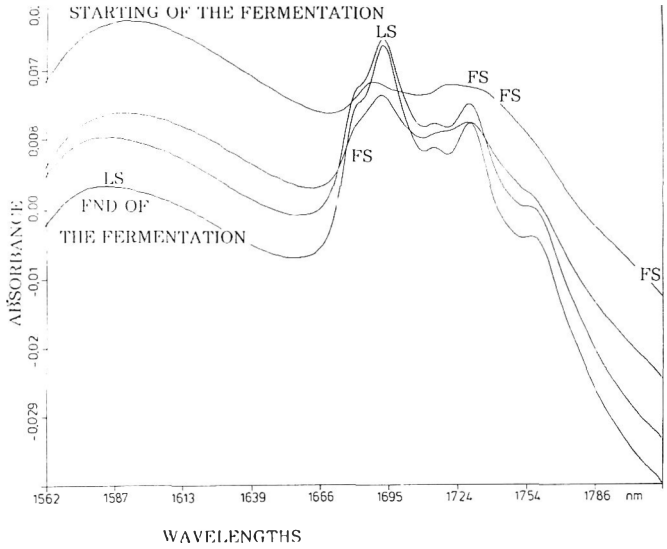

Fig. 1—Infrared Fourier Transform (FTIR) spectrum in the domain of overtones of fermenting musts samples, from starting until end of fermentation (LS = wavelengths of FTIR spectra used for sugar analysis; FS = filters of Technicon 400 used for sugar analysis).

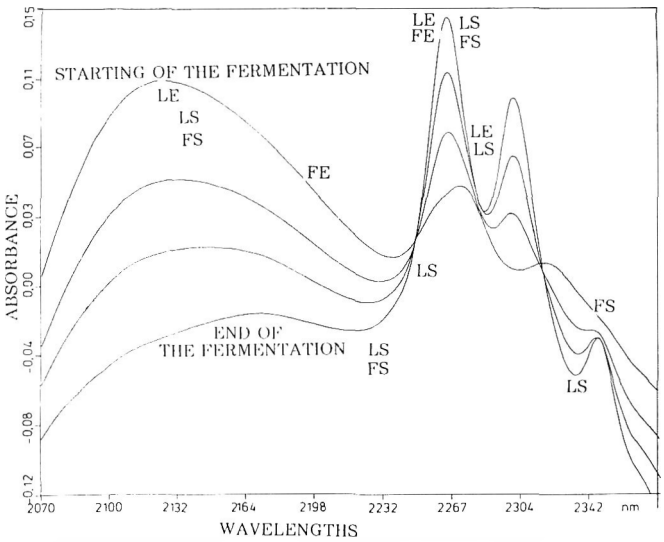

Fig. 2—Infrared Fourier Transform (FTIR) spectrum in the domain of combinations of fermenting musts samples, from starting until end of fermentation (LE and LS = wavelengths of FTIR spectra used for ethanol and sugar analysis respectively; FE and FS = filters of the Technicon 400 used for ethanol and sugar analysis respectively).

RESULTS & DISCUSSION

Calibration of NIR spectrometers

FTIR spectra showed that water presented three main absorption zones in the domain studied, around 1450, 1960, and 2400 nm. Spectral modifications due to the presence of solute appeared between these wavelengths. They corresponded to the vibrations of protons in the groups C-H and O-H, and to the domain of their combinations with C-C or C-O groups. The FTIR wavelengths and the filters which were selected for various regressions appear in Fig. 1 and 2. For ethanol measurement, the regressions concerned essentially the strong absorption zone centered on 2270 nm as indicated in published reports. For sugar determination, the large zone centered around 2130 nm had an amplitude strongly correlated to sugar concentration. The SEC obtained for all fermentations are given in Table 2. The SEC was significantly lower when a larger

Table 2—Alcohol and sugar determinations by NIR spectrometry. SEC for all fermentations (figures in parenthesis indicate number of wavelengths in each correlation).

Components Equipment	Ethanol (vol %)	Sugar (g/l)	
FTIR Spectrometer	0.15	4.6	2.6
	(3[a])	(4[c])	(9[e])
Filter Spectrometer	0.15	4.0	2.4
	(2[b])	(4[d])	(9[f])

[a] 2129-2269-2289 nm
[b] 2207-2270 nm
[c] 1692-2129-2269-2338 nm
[d] 1818-2207-2269-2345 nm
[e] 1595-1692-2129-2243-2269-2281-2289-2309-2338 nm
[f] 1445-1680-1722-1735-1818-2139-2230-2270-2345 nm

number of wavelengths was used, probably because the information about the sugar concentration was spread out in the spectrum.

The specific calibrations for individual fermentations gave lower SEC than when all fermentations were considered: from 0.017 to 0.136 % (v/v) for ethanol and from 0.3 to 1.8 g/L for sugars. The statistical significance of these results was reduced by the limited number of samples per fermentation (15 to 25). In addition, the estimated accuracy was very close to the enzymatic measurements; therefore the discussion is limited to Table 2. For the four calibration fermentations (87 samples), SEC is about 0.15 % (v/v) of ethanol, and 4 g/L or 2.5 g/L of sugar when using four or nine wavelengths, respectively, on either spectrometer.

Calibration of areometers

Table 3 shows the main results of calibrations obtained with density measurements. The fermentation F2 was done in a different way than the others: it had a low (1.077) initial density and a high fermentation temperature (more than 30°C), and ended in 48 hr. Extensive sedimentation was observed at the end as detected by a drop in density at constant ethanol concentration. When taking into account initial conditions of fermentation and considering all fermentations, SEC of about 0.3 % (v/v) ethanol and 4 g/L sugar could be obtained with density as control variable.

Prediction

Two extra fermentations were conducted similarly to F1, respectively with Macabeu (white grapes) and Carignan (red grapes) concentrated musts. Prediction of total sugar and ethanol concentrations for these 2 fermentations had a standard error of 6 g/L sugars and 0.4 % (v/v) ethanol for density, and 2.5 g/L sugars and 0.15 % (v/v) ethanol for each NIR measurement (using 9 filters or wavelengths). Prediction equations were the calibration equations which yielded the lowest SEC in Tables 2 and 3. Predictions were in the same range of magnitude as those cited for density and FIR (El Haloui et al., 1987; Bouvier, 1988). They were improved here, probably because of the similarity between the musts used for calibration and those used for prediction. They were all concentrated juices obtained by evaporation and diluted with water to reconstitute grape juice just before fermentation. This also may be due to the fact that only two fermentations were used for prediction. Consequently, the precise numerical values obtained are not as important as the differences between FIR, FTIR and density.

Comparison of SEC

Without differentiating F2 from others and without taking into account the must initial conditions, NIR spectrometers were as precise as reference enzymatic analyses when carefully selected filters or wavelengths were used. Two or three were enough for ethanol determination, while nine were necessary

Table 3—Alcohol and sugar determinations by areometry. SEC for all fermentations and for all except F2

Components	Ethanol (vol %)		Sugar (g/L)	
Fermentations	all	F1,F3,F4	all	F,F3,F4
s.g.*	1.0	0.61	11.3	5.7
s.g. + $s.g_0$*	0.39	0.17	4.6	3.6
s.g. + S_0**	0.25	0.15	3.6	2.2

* (specific gravity, s.g. and initial specific gravity, $s.g_0$)
** (macrocomponents concentration in initial must)

Table 4—Comparison of alcohol and sugar analyses by NIR spectrometry (FTIR,FIR) and by specific gravity

Components	Ethanol (vol %)		Sugars (g/L)	
Fermentations	all	F1,F3,F4	all	F1,F3,F4
FTIR/FIR	0.06	0.04	1.6	1.5
FTIR/Specific gravity	0.15	0.08	2.3	1.7
FIR/Specific gravity	0.16	0.07	2.7	1.5

for sugars (Table 2). About 16 g/L of sugars were consumed when 1% (v/v) ethanol was produced. Similarly, the SEC of NIR analyses of sugar and ethanol contents were in the same ratio: 16 to 1. This could lead to allowing one estimation of either sugar or ethanol content. Measuring both sugar and ethanol contents gives more detail about the fermentation evolution, because the biochemical ratio of ethanol production versus sugar consumption depends on the physiology of the yeasts.

FTIR and FIR gave a very close SEC for the same number of wavelengths or filters. The strong improvement of signal to noise ratio provided by FTIR in the middle infrared region disappeared in NIR. In the near infrared it was not possible to open the optical diaphragm as widely as required, because of the luminous source and the risk of saturating the detector.

Specific gravity (or density) measurements showed an improved SEC for sugar as well as for ethanol, when F2 is not considered or when initial conditions of fermenting musts are taken into account (Table 3). The ratio between SEC for sugar and SEC for ethanol ranged from 12 to 15. Thus, as density gives only one physical measurement, sugar content is better estimated from it than ethanol content, which can then be deduced by rule of thumb. When considering each fermentation separately, NIR measurements gave a SEC ranging from 0.017 to 0.14 % (v/v) for ethanol and 0.3 to 1.8 g/L for sugar, the bigger SEC corresponded to F2. Results in Table 2 do not make a distinction between all fermentations, and all but F2 fermentations. We expect that, without the F2 data, the SEC of NIR measurements would be less than when all fermentations are considered. Taking F2 fermentation out and taking into account the initial must composition, the SEC of density (Table 3) was as low as that obtained with NIR spectrometry when all fermentations were considered (Table 2). Therefore we confirmed that NIR measurements had an accuracy advantage over density.

Comparison of techniques by least rectangles method

A contribution of comparison accuracies of the NIR and density techniques was made using the least rectangles method (Table 4). Considering all fermentations, FTIR/FIR residuals were 0.06 % (v/v) ethanol and 1.6 g/L sugar. On average, according to practice, 1 % (v/v) of ethanol is produced when 16 g/L sugar is consumed during wine alcoholic fermentation. Therefore, the FTIR and FIR gave closer estimates of ethanol than of sugar contents. Removing F2 data did not significantly change residuals between the two techniques. FTIR/density and FIR/density showed very similar residuals when comparing them for ethanol as well as sugar content estimation considering all fermentations and all except F2. FTIR/FIR residuals for ethanol content estimation were smaller than either FTIR/density or FIR/density residuals when all fermentations were

considered. They were about equal when all but F2 fermentations were considered.

We concluded that least rectangles residuals between techniques were small compared to the accuracy of the reference enzymatic analyses. Also they were smaller than the SEC of each technique for ethanol and sugar content estimation, leading us to suspect the quality of our reference analyses. FIR and FTIR gave very close estimations of ethanol and could be clearly distinguished from density measurements. More experiments are needed to evaluate whether FIR and FTIR are definitely closer to each other than to density for estimation of sugar content. FTIR and FIR gave closer estimates of ethanol than of sugar content. This suggests that they may have a higher accuracy for ethanol than for sugar content. This was already suggested by the necessity of using nine wavelengths or filters for obtaining a satisfactory sugar precision while two or three were enough for ethanol determination.

Comparison of SEP

The SEP of ethanol and sugar for FIR and FTIR were significantly smaller than for density. This meant density was definitely less accurate than NIR techniques. This was not contradictory to the conclusions between NIR and density techniques made through SEC comparison and the least rectangles method, and in full agreement with published findings (El Haloui et al., 1987). This lack of precision of density compared to NIR techniques may be explained by the sensitivity of density to dry extract which composition was variable from one fermentation to another because of differences in cultivars. It reconfirms the need for further work with fresh grape juices.

Synthesis

Density and FIR appeared as two challenging techniques for ethanol and sugar contents estimation during wine alcoholic fermentation. Whenever a 6–8 g/L of sugar and 0.4 to 0.6 % (v/v) ethanol standard of error prediction is enough for production quality, then density is the low-cost method to apply. Whenever a higher accuracy is needed, FIR should be used and will achieve SEP values around 0.15 % (v/v) for ethanol and 2.5 g/L for total sugar. Nevertheless, FTIR applied to the mid infrared region should be investigated for feasibility of gaining more precision. This may be worthy in specific enological conditions where 1 g/L of residual sugar at the end of the fermentation is important to detect.

CONCLUSION

THE EQUIVALENT ACCURACIES in calibration and prediction of both NIR spectrometry techniques showed that the general precision advantages of FTIR disappeared in the NIR. Absorption spectrometers in the NIR have been significantly more precise than areometers for sugar and ethanol content determination during wine alcoholic fermentation. Whenever the prediction accuracy of density for total sugar or ethanol contents is not satisfactory, FIR spectrometry is the recommended alternative for achieving standard errors of prediction as small as 2.5 g/L for total sugar and 0.15% (v/v) for ethanol. While more complex and more costly, but also much more accurate than areometry, FIR spectrometry is a good technological solution for achieving small SEP in predicting total sugar and ethanol during wine alcoholic fermentation.

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Degradation Kinetics of Chlorophylls and Chlorophyllides

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ABSTRACT

Thermal degradation of chlorophylls and chlorophyllides in spinach puree was studied from 100 to 145°C (2–25 min) for chlorophylls and from 80 to 115°C (2.5–39 min) for chlorophyllides. The derivatives formed were: pheophorbides, pyropheophorbides, pheophytins and pyropheophytins. Degradation kinetics of chlorophylls and chlorophyllides followed a first-order kinetic model. Reaction rate data showed that the *a* form of both chlorophylls and chlorophyllides degraded more rapidly than the *b* form. Chlorophyllides were less stable than chlorophylls. Activation energies ranged from 15.0 to 22.8 Kcal/mol. A kinetic compensation effect was observed for both chlorophylls and chlorophyllides with an isokinetic temperature of 160.8°C. The relative stability of these compounds suggests that methods to maximize chlorophyllides would not be effective for improving green color stability.

Key Words: degradation, kinetics, vegetables, chlorophyll, chlorophyllides, spinach

INTRODUCTION

THERMALLY PROCESSED green vegetables exhibit poor color quality as compared with fresh ones. Researchers (Mackinney and Weast 1940; Gold and Weckel, 1959) have reported that color changes (from bright green to olive-brown) occurring during processing could be attributed mostly to conversion of chlorophylls to pheophytins. Blanching of vegetables has been shown to induce formation of C-10 epimers designated as chlorophylls *a'* and *b'* (Katz et al. 1968). During prolonged heat treatments such as canning, chlorophylls are converted to pheophytins *a* and *b*. In addition, the formation of C-10 decarbomethoxylated derivatives known as pyropheophytins *a* and *b*, may occur (Pennington et al., 1964; Schwartz et al., 1981).

The kinetics of chlorophyll degradation has been studied extensively. Studies at different temperatures showed the rate of this degradation followed a first-order kinetic model (Mackinney and Joslyn, 1941). Lajollo et al. (1971) reported that chlorophyll *a* degraded 2.5 times faster than chlorophyll *b* at 37°C and at a water activity of 0.32. During storage of aseptically processed spinach puree, Schwartz and Lorenzo (1991) showed that the *a* form degraded 4 to 10 times faster than the *b* form. Other researchers also reported the greater susceptibility to degradation of the *a* form with respect to the *b* form (Tan and Francis, 1962; Buckle and Edwards, 1970; Schwartz and von Elbe, 1983).

Various methods to preserve green color of vegetables have been proposed. Control has been used to maintain pH at high levels during heat treatments and storage. This procedure was beneficial after processing but eventually during storage the conversion of chlorophylls to pheophytins occurs, causing dramatic color changes (Malecki, 1964). High-temperature short-time (HTST) treatments have been used to preserve green color. HTST yields good results immediately after processing, but a rapid chlorophyll degradation occurs during storage (Tan and Francis, 1962). Reduction of water activity in dried products can lower the degradation rate of chlorophylls to pheophytins (Dutton et al., 1943; Lajollo et al., 1971). Addition of metals such as copper and zinc have been used to form a pheophytin-metal complex with a regreening effect on processed vegetables (Schanderl et al., 1965; Jones et al., 1977; von Elbe, 1989). Assuming that green chlorophyllides were more stable than chlorophylls, a process which promotes conversion of chlorophylls to chlorophyllides (via chlorophyllase) has been proposed to enhance green color of canned vegetables (Borodin, 1882; Thomas, 1928; Lesley and Shumate, 1937). Clydesdale and Francis (1968) produced chlorophyllides in plant tissue by activating the enzyme chlorophyllase. Preservation or improvement of green color in the product was minimal, because of the small amount of chlorophyllides produced. Chlorophyllides were reported to be slightly more stable than chlorophylls. However, their studies were not conclusive because of the difficulty in producing large quantities of chlorophyllides and the lack of a simple and sensitive separation technique to monitor their degradation.

Our study describes the kinetics of degradation of chlorophylls and chlorophyllides at various processing conditions in order to determine the stability of the different compounds during thermal treatments. Kinetic parameters such as reaction order, reaction rate constants and activation energies were determined to define degradation reactions.

MATERIALS & METHODS

Sample preparation

Fresh spinach (*Spinacia oleracea*, melody variety) from local markets was used. It was washed, drained and chopped in small pieces (about 1 cm^2). Spinach leaves (30g) were weighed in a 500 mL Erlenmeyer flask, covered with 70g of water and incubated at 65°C for 30 min in a water bath (Haake A81, Berlin, West Germany) to activate endogenous chlorophyllase. After incubation, the mixture was cooled to room temperature, and blended for 4 min resulting in a homogeneous puree. After blending, the puree was deaerated in a glass chamber using vacuum. A batch of 4 kg of puree was prepared, quick-frozen in 25 mL test tubes using liquid nitrogen, and kept frozen (−20°C) until use. Samples were thawed immediately before thermal processing.

Extraction of pigments

Spinach puree (5 g) was weighed in a 25 mL Erlenmeyer flask, and 18.8 mL of acetone added. The mixture was ground in a Tekmar tissumizer, model TR-10Z (Tekmar Co., Cincinnati, OH) for 2 min, filtered through Whatman # 1 and Whatman # 42 filter paper and brought to volume with solvent (80:20, acetone-water) in a 25 mL volumetric flask. (Schwartz et al., 1981). Before injecting 30 μL of the sample onto the HPLC column, the extract was filtered through a 0.45 μm nylon syringe filter (Micron Separations Inc., Westboro, MA). Two replicate extractions of each sample were performed and each extract was analyzed by HPLC in duplicate.

HPLC conditions

The HPLC system consisted of a C-18 column, 5 μm particles 4.6 mm i.d. × 25 cm (MacMod Analytical Inc., Chadds Ford, PA), an U6K injector and two model 510 pumps (Waters Associates, Milford, MA). Pigments were selectively screened at 658 nm using a Linear UVIS Model 203 detector (Anspec Co., Inc., Ann Arbor, MI). The gradient system of solvents consisted of ethyl acetate/methanol/water as follows: solvent A, 15/65/20, and solvent B, 60/30/10. The gradient

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profile was controlled using a gradient controller model 680 (Waters Associates, Milford, MA) as described by Canjura and Schwartz (1991).

Chromatographic peak data

Chromatographic data was collected on an Apple II+ computer equipped with an ADALAB data acquisition/control card. The area under the peaks was evaluated using commercial software CHROMATOCHART-AP version 2.16 (Interactive Microware, Inc., State College, PA).

Thermal treatments

Glass tubes (0.98 mm external diameter, 1.38 cm length) were constructed using the method described by Nunes and Swartzel (1990). The tubes had a capacity to hold 1.5 to 2.0 mL of puree. Samples were injected in the tubes with a 50 mL plastic syringe equipped with 15G (stainless steel) needle and sealed using a double jet burner oxygen-propane torch for rapid sealing. The tubes containing the sample were kept in an ice-water bath during sealing to avoid heating the spinach puree prior to treatment. Thermal treatments of samples were performed by immersing the tubes in an oil bath MGU Lauda (Brinkmann Instruments, West-Germany). At each time-temperature combination, seven tubes were processed. After processing, samples from each treatment were combined, extracted and analyzed by HPLC to determine the content of chlorophylls and derivatives. For the kinetic analysis, experimental conditions were established to provide a wide temperature range of heating for chlorophylls 100–145°C and chlorophyllides 80–115°C. With increasing processing temperature range, the confidence interval has been shown to become smaller and determination of kinetic parameters are more precise (Lund, 1983). Temperatures and times of treatment were as follows: For chlorophyllides: Temp. °C (time in min), 80°C (9, 15, 21, 27, 33, 39); 90°C (3, 6, 9, 12, 15, 18); 100°C (2.5, 3, 4.5, 6, 7, 8); 115°C (2.5, 3, 3.5, 4, 5, 7, 10). For chlorophylls: 100°C (3, 6, 9, 12, 20, 25); 115°C (2.5, 4, 5, 6, 8); 130°C (2, 3, 4, 6, 8, 10); 145°C (2, 2.5, 3, 3.5, 4).

Time-temperature data acquisition

Two type T thermocouples were constructed and placed in a tube containing spinach sample, one outside (fast response) and the other inside. To avoid wall heating effects the internal thermocouple was carefully placed at the center of the tube (Nunes and Swartzel, 1990). The top part of the tube was sealed with silicone glue. Seals were previously tested at high temperature (145°C) to detect leaks. The thermocouples were calibrated between 10 and 145°C; therefore, temperatures to estimate kinetic parameters were corrected accordingly. Time-temperature curves for heating, holding and cooling were registered for each time-temperature combination connecting the thermocouples to a DASH-8 data acquisition and control interface board (MetraByte Corporation, Tauton, MA) and an IBM personal computer running with a data acquisition software Labtechnotebook System version 3.0 (Laboratory Technologies Corporation, Wilmington, MA).

Kinetic data analysis

A modified steady-state procedure was used in which data were collected only in the isothermic portion of heating. Samples were heated to reach the processing temperature and held for the desired time (Nunes and Swartzel, 1991). A traditional two-step method was used to calculate activation energies. This method of analysis required plotting the log percent of compound remaining vs time:

$$\ln (C) = \ln(C_0) + k (T_i)\, t_i + J_i \qquad (1)$$

to fit a kinetic model and to obtain the reaction rate constants from regression lines.

The activation energies were evaluated using the Arrhenius equation:

$$\ln k = - E_a/RT + \ln k_o \qquad (2)$$

where E_a is activation energy, R the universal gas constant and k_o the frequency factor. Regressing the logarithm of the rate constants vs 1/T, the slope and intercept were obtained using least squares linear regression. From the slope of the line, E_a/R was obtained. Enthalpy (ΔH^*) and entropy (ΔS^*) were obtained by plotting the equation derived from transition state theory, i.e.,

$$\ln (k/T) = \ln (k_B/h) + \Delta S^*/R - \Delta H^*/R\ (1/T) \qquad (3)$$

where k_B is Boltzman's constant and h is Planck's constant. From the slope and intercept of this line ΔH^* and ΔS^* respectively were obtained.

A compensation effect can be linearly expressed by a relationship between enthalpy (ΔH^*) and entropy (ΔS^*), which compensate each other, to produce small changes in the free energy of a reaction. Another way to express compensation is use of kinetic parameters such as activation energy (E_a) and frequency factor (k_o) (Zsakó, 1976; Garn, 1976; Rhim et al., 1989). For a series of reactions, the change in activation energy is accompanied by the parallel increase of the frequency factor:

$$\ln k_o = AE_a + B \qquad (4)$$

where $A = 1/RT_{iso}$. T_{iso} is known as the isokinetic temperature, characteristic of a family of reactions and is not an experimental temperature. At this temperature, reaction rate constants are equal for all reactions involved. Some researchers (Krug et al., 1976a,b) have shown that under certain conditions compensation effect may occur accidentally as a computational artifact from experimental errors. To test the validity of the compensation effect for a reaction, a statistical method has been developed, which consists of comparing the isokinetic temperature, T_{iso} with the harmonic mean temperature T_{hm}. The harmonic temperature is defined by:

$$T_{hm} = n/\textstyle\sum_{i=1}^{n} (1/T_i) \qquad (5)$$

if they are significantly different ($T_{iso} \neq T_{hm}$), the existence of true compensation is suggested for the reactions involved (Krug et al., 1976a,b; Rhim et al., 1989).

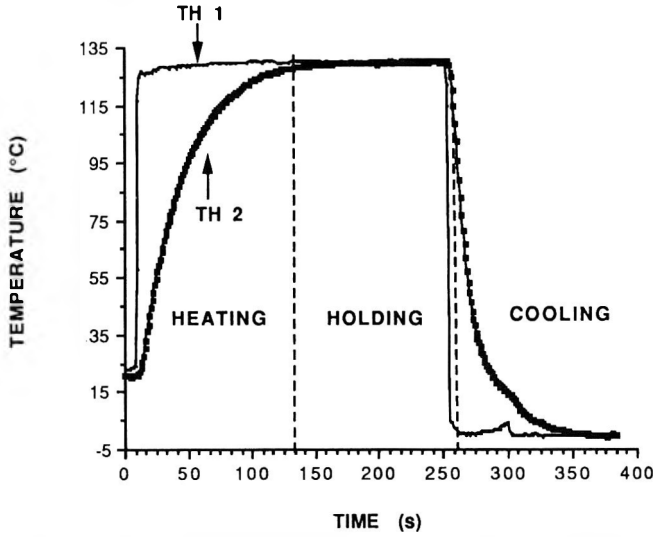

Fig. 1—Time-temperature curve for sample treated at 130°C for 4 min. TH 1, thermocouple located outside of tube. TH 2, thermocouple located at center of tube.

RESULTS & DISCUSSION

FIGURE 1 SHOWS a typical time-temperature curve for a sample from room temperature to 130°C, held for 4 min and cooled in an ice-water bath. Thin-wall glass tubes were employed to minimize the time to reach processing temperature. The come-up time at each processing temperature was 140 ± 4 sec. Figure 2 shows typical HPLC chromatograms for spinach puree heated at 100°C for 3 min and at 145°C for 2 min. These chromatograms showed chlorophyll and its derivatives and indicated that thermal treatments induced chlorophyll degradation to form pheophytins and pyropheophytins. Similarly, chlorophyllides degraded to form pheophorbides and pyropheophorbides. The degradation products were in agreement

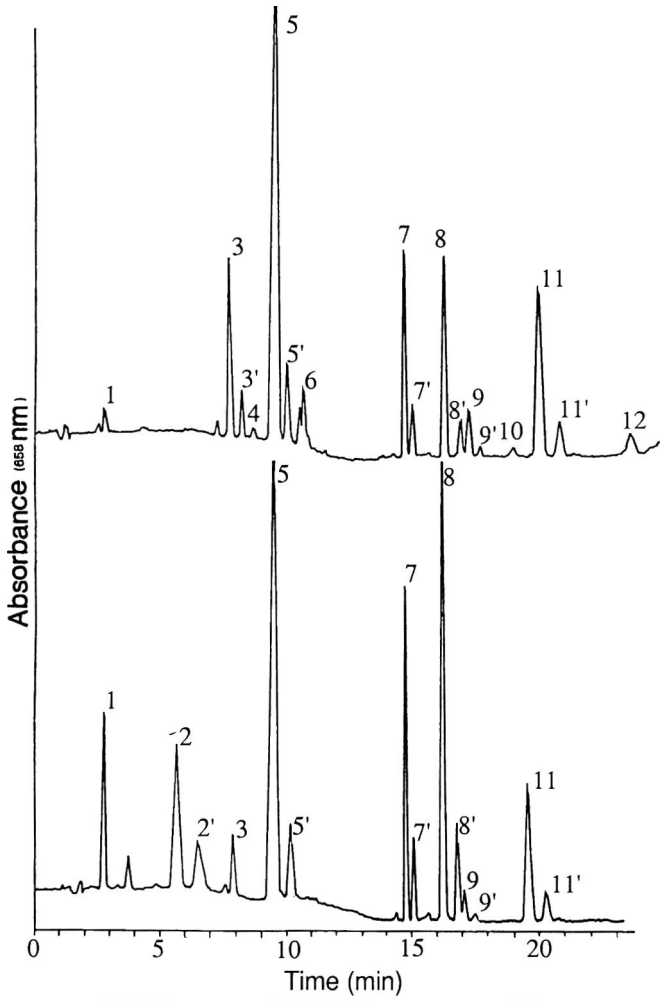

Fig. 2—HPLC chromatograms of chlorophyll derivatives in heat processed spinach puree. [Top (145°C, 2 min); Bottom (100°C, 3 min)] Peak 1=chlorophyllide b', *peak 2=chlorophyllide* a, *peak 2'=chlorophyllide* a', *peak 3=pheophorbide* b, *peak 3'=pheophorbide* b', *peak 4=pyropheophorbide* b, *peak 5=pheophorbide* a, *peak 5'=pheophorbide* a', *peak 6=pyropheophorbide* a, *peak 7=chlorophyll* b, *peak 7'=chlorophyll* b', *peak 8=chlorophyll* a, *peak 8'=chlorophyll* a', *peak 9=pheophytin* b, *peak 9'=pheophytin* b', *peak 10=pyropheophytin* b, *peak 11=pheophytin* a' *peak 11'=pheophytin* a' *and peak 12=pyropheophytin* a.

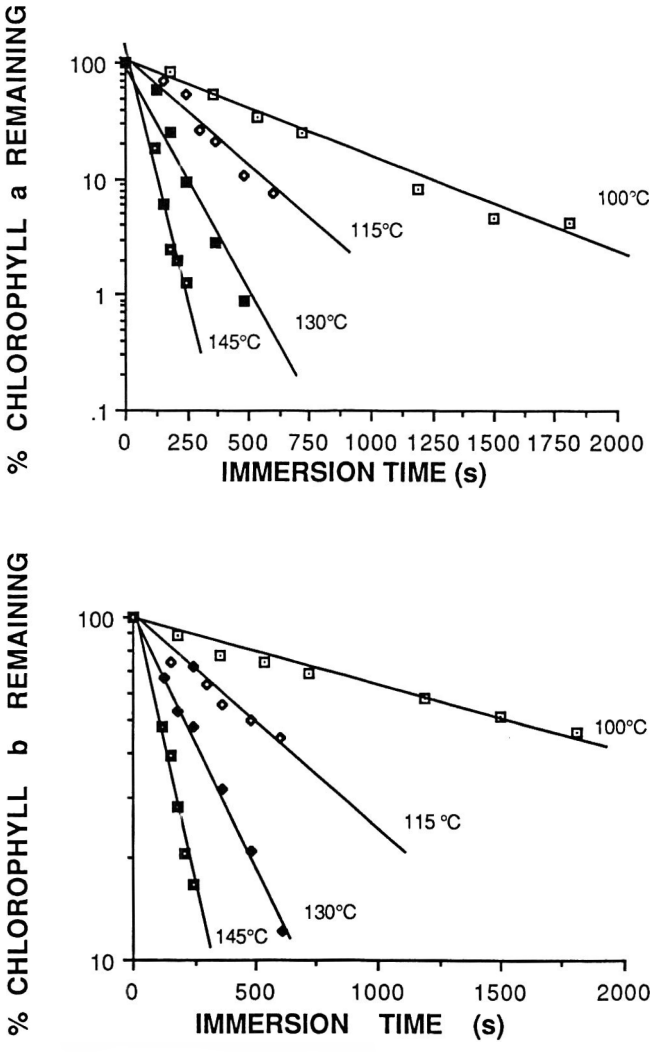

Fig. 3—Chlorophyll degradation rates in heat processed spinach puree.

with previous findings (Mackinney and Joslyn 1941; Lajollo et al., 1971; Schwartz et al., 1983).

Using the HPLC separation technique, 12 chlorophyll derivatives were resolved, but only four components were quantitated for the kinetic analysis (chlorophylls *a, b* and chlorophyllides *a, b*). The kinetic study was based on degradation of chlorophylls and chlorophyllides, rather than on formation of degradative compounds. In Fig. 3, the percent of chlorophyll *a* and *b* remaining vs. immersion time is plotted on a semilogarithmic scale. From the slope of this line, the reaction rate constant (k) was obtained using linear regression for each processing temperature. The straight lines confirmed that degradation reactions of chlorophylls *a* and *b* followed a first-order reaction kinetic model, i.e., $\ln (C) = k t + \ln (Co)$. Results agreed with reported data (Gold and Weckel, 1959; Gupte et al., 1964; Schwartz et al., 1983).

Figure 4 shows degradation curves for chlorophyllides plotted as a percent of chlorophyllide remaining vs. immersion time. A linear relationship was obtained demonstrating that chlorophyllides *a* and *b* followed a first-order kinetic model. Table 1 lists the reaction rate constants and the correlation coefficients for degradation of chlorophylls and chlorophyllides with their respective standard deviations at different temperatures. The relative degradation of chlorophyll *a* with respect to *b* showed that chlorophyll *a* degraded 2 to 6 times faster than chlorophyll *b* depending on temperature. Results were in agreement with previous reports. Lajollo et al. (1971) reported that chlorophyll *a* degraded 2.5 times faster than chlorophyll *b*, while Schwartz and Lorenzo (1991) reported this ratio to be 4 to 10 times in spinach puree during storage. A similar trend was found in our study for relative degradation rates of chlorophyllide *a* which degraded 1 to 3 times faster than chlorophyllide *b* at processing temperatures 80 to 115°C. At higher temperatures, differences were less pronounced. Consequently, less decomposition of both chlorophyll *a* and chlorophyllide *a* occurred during HTST treatments suggesting this treatment could be used to maximize pigment content after processing.

Comparing reaction rate data at 115°C, chlorophyllide *a* degraded 2.0 times faster than chlorophyll *a* and chlorophyllide *b* degraded 4.0 times faster than chlorophyll *b*. However, food products are generally processed at temperatures above 115°C. Heating of chlorophyllides was conducted at lower temperatures because of greater susceptibility to thermal treatments. In order to compare the relative thermal instability of chlorophyllides compared to chlorophylls, an extrapolation of lines in the Arrhenius plot was performed. We assumed that activation energy would be constant within the experimental temperature range. This yielded the reaction rate constants at 130 and 145°C. Further differences were noticed when comparing

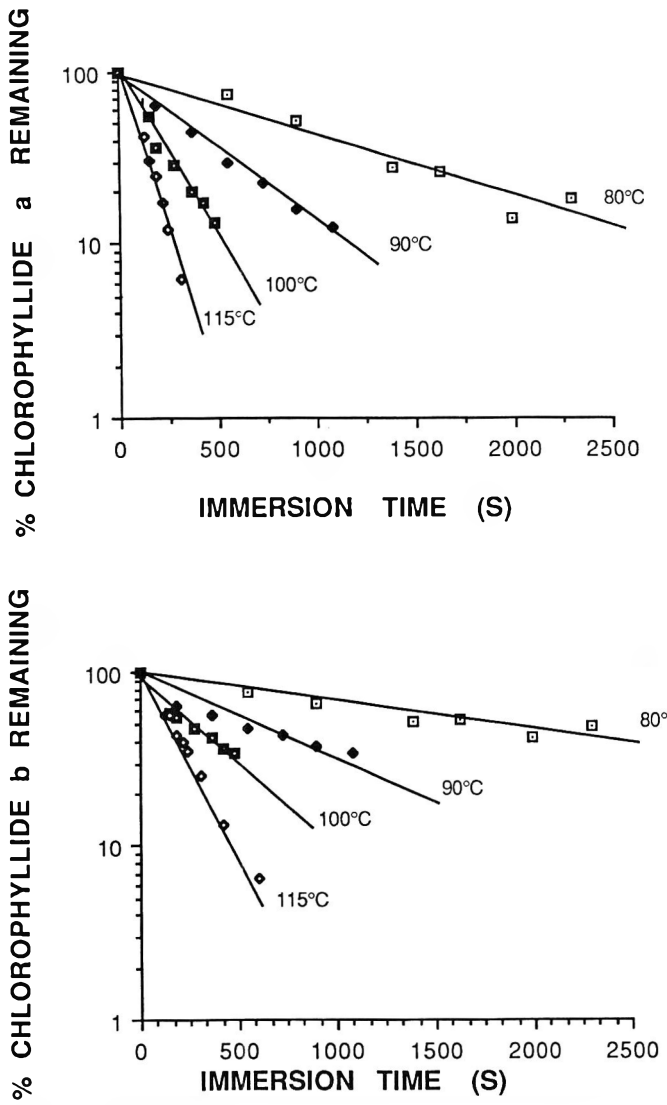

Fig 4—Chlorophyllide degradation rates in heat processed spinach puree.

Table 1—Reaction rate constants and correlation coefficients of chlorophylls a, b, *and chlorophyllides* a, b *degradation*

Temperature (°C)	Form *a* (k ± sd) × 10^{-3} (1/sec)	Correlation coefficient	Form *b* (k ± sd) × 10^{-3} (1/sec)	Correlation coefficient
Chlorophyll				
100	2.2 ± 0.1	0.99	0.4 ± 0.0	1.00
115	5.2 ± 0.6	0.98	1.2 ± 0.1	0.98
130	11.9 ± 0.7	0.99	3.5 ± 0.2	1.00
145	19.3 ± 3.5	0.97	9.8 ± 1.0	0.99
Chlorophyllide				
80	0.9 ± 0.2	0.95	0.3 ± 0.1	0.92
90	1.8 ± 0.1	1.00	0.7 ± 0.1	0.99
100	4.2 ± 0.3	0.99	1.7 ± 0.1	1.00
115	10.1 ± 1.3	0.99	5.0 ± 0.3	0.99

these values relative to reaction rates found for chlorophylls *a* and *b* at the same temperatures. Chlorophyllide *a* degraded 2.0 to 3.7 times faster than chlorophyll *a* and chlorophyllide *b* 4.1 to 4.9 times faster than chlorophyll *b* at 130 and 145°C, respectively. These results confirmed that chlorophyllides are more labile to thermal treatments than chlorophylls which is in disagreement with some reported suggestions (Borodin, 1882; Thomas, 1928; Lesley and Shumate, 1937; Clydesdale and Francis, 1968). The difference between our experimental data and published reports may be due to the successful activation of the enzyme chlorophyllase, the sensitive HPLC separation

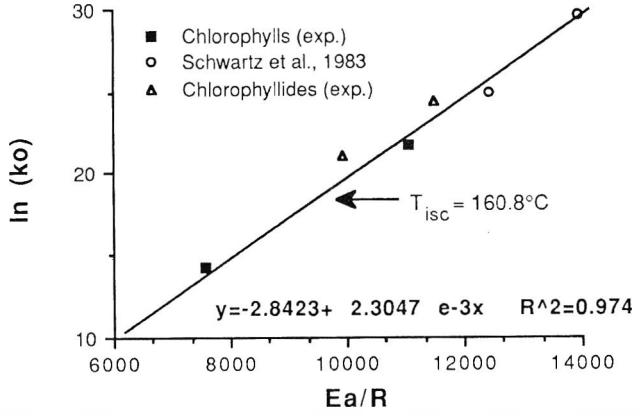

Fig. 5—Kinetic compensation effect of chlorophylls and chlorophyllides.

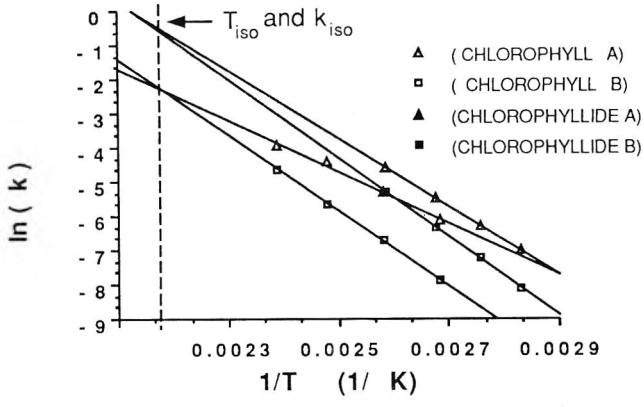

Fig. 6—Arrhenius plot for chlorophylls and chlorophyllides.

Table 2—Activation energy, frequency factor, enthalpy and entropy for chlorophylls and chlorophyllides

Compound	Ea[a] (two step method) (Kcal/mol	Intercept (ln Ko)[a]	Correlation coefficeint[1]	ΔS*[b] (Cal/mol K)	ΔH*[b] (Kcal/mol
Chlorophyll					
a	15.0 ± 0.9	14.2	0.995	−33.0	14.2 ± 1.0
b	22.0 ± 0.3	21.8	0.999	−18.6	20.9 ± 0.2
Chlorophyllide					
a	19.7 ± 0.1	21.1	0.999	−20.7	18.4 ± 0.7
b	22.8 ± 0.4	24.4	0.999	−0.87	22.0 ± 0.4

[a] Values obtained from the slopes of Arrhenius plot.
[b] Values obtained from transition state theory equations.

technique used and the kinetic analysis we performed. Further, water soluble chlorophyllides are more likely to contact the protic aqueous environment more so than the lipid soluble chlorophylls. Thus, degradation due to loss of Mg from the porphyrin ring and displacement by two protons may be a more favored reaction with water-soluble compounds such as chlorophyllides.

Previous studies on spinach puree reported a wide range of activation energies for degradation of chlorophyll *a* and *b*. For chlorophyll *a*, they ranged from 12.5 to 25.2 (Kcal/mol) and for chlorophyll *b*, from 7.5 to 22.5 (Kcal/mol, Mackinney and Joslyn, 1941; Gupte et al., 1964; Herrmann, 1974; Schwartz et al., 1983). Table 2 lists activation energies with respective standard deviations and correlation coefficients, frequency factors, entropy and enthalpy of chlorophylls and chlorophyllides. Activation energies for chlorophyll degradation are in agreement with reported ranges (Mackinney and Joslyn, 1941; Gupte et el., 1964; Schwartz et al., 1983). The activation energy for chlorophyll *a* (15.0 Kcal/mol) was less than that for chlorophyll *b* (22.0 Kcal/mol). This was in contrast to previously

reported values which indicated the activation energy of the *b* form was less than that of the *a* form (Gupte et al., 1964; Herrmann, 1974; Schwartz et al., 1983). These variations between reported values and experimental values may be attributed to different sample materials with varying pH values, different temperature ranges, and different analytical methods used to quantify pigments. Activation energies of chlorophyllides *a* and *b* were higher than those of chlorophylls. A higher activation energy implies that a smaller temperature change is needed to degrade a specific compound more rapidly. Therefore, chlorophyllides are more susceptible to thermal degradation than chlorophylls.

Figure 5 illustrates a plot of ln (k_o) vs. E_a/R which indicates linear compensation between these kinetic parameters. This plot includes our four experimental values and two reported values (Schwartz et al., 1983). The isokinetic temperature (T_{iso}) determine from the slope of the line was 160.8°C ($r^2 = 0.974$) while the mean harmonic temperature (T_{hm}, see formula 5) was calculated as 112°C. Consequently, $T_{iso} \neq T_{hm}$ which suggests existence of a true compensation effect for degradation of chlorophylls and chlorophyllides (Krug et al., 1976 a,b). Extrapolating the lines in the Arrhenius plot (Figure 6), two intersection points were observed, which yielded similar T_{iso}, but unexpectedly different k_{iso} (reaction rate at T_{iso}) for degradation of both chlorophylls and chlorophyllides. Reactions which exhibit compensation but show different reaction rates at the isokinetic temperature have been reported previously (Ranganathan et al., 1977). To explain this for chlorophyll and chlorophyllide degradation, further research is needed to study the significance of different parallel compensation lines and their relationship to physical and chemical properties. Among other factors, the study of chlorophylls and chlorophyllides degradation at different pH values may be useful to demonstrate the existence of a true compensation effect on degradation of these compounds.

CONCLUSIONS

CONTRARY to previous reports in spinach puree, chlorophyllides were found less stable than chlorophylls during thermal processing. Therefore, procedures to maximize chlorophyllide formation from chlorophyll would not be effective in maintaining optimum green color. Thus, vegetables containing chlorophyllides would be expected to degrade faster during storage than those containing chlorophylls. However other vegetable products need to be tested to confirm this conclusion. Existence of an apparent compensation effect for degradation of chlorophylls and chlorophyllides suggested that these compounds degrade by similar mechanisms, however further studies are needed to provide more conclusive results.

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Lipid Changes during Frying of Frozen Prefried Foods

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ABSTRACT

Fat absorption, lipid interchange and preferential adsorption of polar compounds on food surfaces were studied during frying of frozen prefried foods. Four frying oils were used to fry frozen potatoes and battered hake. Major and minor changes in food fats and frying oils were determined. Lipid determination after frying demonstrated significant differences in fat absorption due to the physical structure of foods. From quantitation of major fatty acids, lipid interchange between frying oils and food fats could be determined. Polar compound concentrations and distributions demonstrated no preferential adsorption on food surfaces during frying of these prefried foods.

Key Words: frying frozen-foods, prefried, lipids, fatty-acids fats/oils

INTRODUCTION

THE INCREASE in the consumption of frozen foods is promoting development of new products of high added value characterized by the presence of used frying fat in their composition. Such frozen pre-fried foods need to be prepared before ingestion; one of the most common methods of cooking being deep-fat frying (Haumann, 1987). Several differences between prefried food frying and fresh food frying are notable. Prefried food fat usually contains a significant amount of polar compounds which can have an influence on final food quality and on subsequent behavior of frying oils. The type of oil or fat used for prefrying mainly depends on availability and price (Orthoefer, 1987). Therefore, the composition of some fried food fat may be unpredictable. Finally, dilution of prefried food fat in frying oil must be much higher than in fresh food frying since the fat is found in the surface layers of the fried substrate (Pokorny, 1980).

Various interesting previous studies on frying of prefried potatoes have been carried out. Toma et al. (1986) reported the influence of the food surface treatment on oil absorption. Degradation of frying fats has been determined using different fats and conditions. Results have suggested the suitability of fats with low unsaturation (Keijbets et al., 1985, 1986). They also show the necessity of controlling two variables — amount of fried food and replenishment of oil in the fryer — in order to maintain quality of oils (Thompson and Aust, 1983).

With respect to lipid interchange, Sebedio et al. (1990) found similar levels of polar components and polymers in both fried potatoes and frying oils. Aust and Thompson (1981) reported that composition of fried potatoes resembled that of the frying oil, due to a big increase in oil content during frying. Nevertheless, quantitative data on lipid changes in both directions - food fat released to the frying oil and frying oil retained by the food - have not been reported. Also references on frying of prefried foods other than potatoes have not been found.

The objective of our study was to determine the influence of both degree of unsaturation and alteration level of frying oils, as well as type of food, on lipid interchange and preferential adsorption of fat on food surfaces. For this purpose, a mathematical calculation was applied to determine the level of lipid interchange. Furthermore, a new approach was used to provide information on preferential adsorption on food surfaces.

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MATERIALS & METHODS

Initial samples

Four frying oils (olive and sunflower, both without heating and after discarding in domestic frying) were used to fry commercially available frozen prefried potatoes and battered hake. Fresh oils were supplied by an industrial manufacturer. The discarded oils came from previous experiments carried out with the collaboration of 10 housewives (Pérez-Camino et al., 1987). For industrial processing of prefried potatoes slightly hydrogenated palm oil was used while the prefried hake was prepared with sunflower oil. Chemical characteristics of initial oils and of the lipids extracted from prefried foods are given in Tables 1 and 2.

Frying procedure

Eight fryers (4 oils × 2 foods) each containing 1L of oil were used to prepare 1.2 kg (6 lots × 200g) of prefried food in each lot. Temperature was held at 185°C and a 6 min period was established for each operation after which samples of 2g were taken. At the end of the experiment, the 52 samples of frying oils and 18 samples of lipids extracted from prefried foods and from fried food after the first and last frying, were packaged under nitrogen and stored at −25°C until required.

Analytical methods

Moisture of prefried and fried foods was determined using the vacuum oven method (AOAC, 1990). Food lipids were obtained by 5 hr Sohxlet extraction (AENOR, 1980), using diethyl ether as solvent. Dry lipid-free matter was calculated by subtracting moisture and lipid contents of each sample from 100.

Quantitative analysis of fatty acids was carried out by gas liquid chromatography (GLC) following transesterification of the samples with Ch_3ONa and $HCl-CH_3OH$. The column was 2m long and 0.32 cm i.d. packed with 15% diethylene glycol succinate (DEGS) on Supelcoport 80-100 mesh at 180°C. Methyl heptadecanoate was used as internal standard (Dobarganes and Pérez-Camino, 1988).

Concentrations and distribution of polar compounds were deter-

Table 1—Quantitation of polar compounds in the initial frying oil and in lipids from prefried foods (mg/g)

Initial samples	Total polar compounds[a] (% fat basis)	Distribution of polar compounds (mg/g)[b]			
		TG P	ox TG	DG	FA
Unheated olive oil	4.8	3.9	7.6	30.4	5.9
	(0.21)	(0.25)	(0.89)	(1.37)	(0.48)
Used olive oil	18.6	62.3	69.9	44.0	10.1
	(0.40)	(1.30)	(1.52)	(2.69)	(0.40)
Unheated sunflower oil	4.8	10.8	18.1	13.0	6.0
	(0.18)	(0.97)	(1.31)	(1.36)	(0.55)
Used sunflower oil	23.2	124.8	78.3	21.9	7.3
	(0.21)	(1.46)	(1.87)	(1.39)	(0.45)
Prefried potato lipids	19.6	42.4	63.5	84.4	5.3
	(0.50)	(1.37)	(0.89)	(3.42)	(0.25)
Prefried battered hake lipids	13.9	55.0	46.8	24.5	12.7
	(0.53)	(1.11)	(2.04)	(1.85)	(0.47)

[a] Means of three determinations.
Numbers within brackets are standard deviations (S_x).

[b] Abbreviations: TG P -Triglyceride polymers; ox TG -Oxidized triglyceride monomers; DG -Diglycerides; FA -Fatty acids.

Table 2—Quantitation of major fatty acids before and after first frying (wt% on fat basis)

	Sample	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	Other fatty acids	Total nonaltered fatty acids
Before frying	Unheated olive oil	10.4	3.2	76.3	8.3	0.7	98.9
	Used olive oil	11.4	3.6	71.2	7.7	1.0	94.9
	Unheated sunflower oil	6.9	4.3	22.2	65.2	0.2	98.8
	Used sunflower oil	8.1	4.5	23.3	56.2	0.7	92.8
	Prefried potato lipids	43.4	6.8	40.9	2.9	1.2	95.2
	Prefried battered hake lipids	7.1	4.4	28.9	52.8	1.6	94.8
Potato lipids after 1st frying	Unheated olive oil	12.4	3.5	73.4	7.9	0.9	98.1
	Used olive oil	13.8	3.9	68.4	7.3	1.3	94.7
	Unheated sunflower oil	9.5	4.6	24.7	58.9	0.1	97.8
	Used sunflower oil	10.8	5.0	25.0	51.8	1.0	93.6
Battered hake lipids after 1st frying	Unheated olive oil	10.3	3.3	72.3	11.5	0.9	98.3
	Used olive oil	11.1	3.7	68.0	10.6	1.0	94.4
	Unheated sunflower oil	7.0	4.0	23.1	63.7	0.2	98.0
	Used sunflower oil	7.8	4.8	24.2	55.3	1.0	93.1

Table 3—Concentrations (%) of frying oils in food lipids after first frying

		Application of Eq. (1) to significant fatty acids				
Prefried foods	Sample	$C_{16:0}$	$C_{18:1}$	$C_{18:2}$	$\bar{x}$[a]	$S_{\bar{x}}$[b]
Potatoes	Unheated olive oil	93.9	91.8	–	92.8	1.48
	Used olive oil	92.5	90.8	–	91.7	1.20
	Unheated sunflower oil	92.9	–	89.9	91.4	2.12
	Used sunflower oil	92.4	–	91.7	92.1	0.49
Battered hake	Unheated olive oil	–	91.6	92.8	92.2	0.85
	Used olive oil	–	92.4	93.6	93.0	0.85

[a] Means of the two determinations
[b] Standard deviations

mined in initial samples and in frying oils and food lipids after the 1st and 6th fryings. Polar compounds were determined by silica column chromatography, following the method proposed by the IUPAC (Waltking and Wessels, 1981), with two slight modifications: hexane:ethyl ether (90:10) was used to elute the nonpolar fraction to obtain a sharper separation (Dobarganes et al., 1984; Perrin et al., 1984) and a final elution of the column was made with CH_3OH to improve recovery of the sample (Billek et al., 1978; Grandgirard and Julliard, 1984). Secondly, distribution of polar compounds was performed by high performance size exclusion chromatography (HPSEC) (Dobarganes et al., 1988).

Samples were analyzed in a Konik Model 500 A chromatograph (Konik S.A., Barcelona, Spain), with a 10 µL sample loop, a Hewlett Packard 1037 A refractive index detector (Hewlett Packard, Pittsburgh, PA, USA) and two 100 Å and 500 Å Ultrastyragel columns (Waters Associates, Milford, MA), connected in series and operated at 35 °C. The columns were 25 cm × 0.77 cm i.d., packed with porous, highly cross-linked styrenedivinylbenzene copolymer (≤ 10 µm). HPLC-grade tetrahydrofuran served as the mobile phase with a flow-rate of 1 mL/min and the sample concentration was between 15 and 20 mg/mL in tetrahydrofuran.

RESULTS & DISCUSSION

TABLE 1 and the upper part of Table 2 summarize the chemical characteristics of the initial frying oils and of the lipids extracted from the prefried foods. The fresh oils and those used before hand in frying differed clearly in level of altered fatty acids, although the polar compounds were in no case higher than the 25–30% suggested in some countries as criteria for discarding the oil (Castang, 1981; Meyer, 1979; Dobarganes et al., 1989). At the same time, the selection of olive and sunflower oils included samples of different degrees of unsaturation. In relation to the lipids extracted from the prefried foods, in both cases the amount of polar compounds was also lower than 25%.

The polar compound distribution determined by means of HPSEC allowed differentiation of the main groups of compounds present and establishment of the relative influence of the three degradation pathways predominant in frying: hydrolysis, polymerization and oxidation. Total polar compounds and polar compound distribution were analyzed in triplicate in the initial fats. Reproducibility was excellent for total polar compound determination, the coefficient of variation being less than 4.4%. With respect to polar compound distribution, diglycerides are usually determined with the highest error by this procedure, though the coefficient of variation was always lower than 10.5%.

Despite the similar concentrations of polar compounds in

Table 4—Major changes during the frying of prefried foods (wt% on prefried food basis)

			After frying			
Frozen prefried foods	Analysis	Before frying	Unheated olive oil	Used olive oil	Unheated sunflower oil	Used sunflower oil
Potatoes	Moisture	66.9[a]	23.2	24.4	23.1	23.6
			(0.34)	(0.50)	(0.43)	(0.40)	(0.46)
	Lipids	4.6	8.5	8.3	9.1	8.3
		(0.11)	(0.28)	(0.82)	(0.24)	(0.39)
	Dry, lipid-free matter	28.5	29.9	28.5	28.4	30.0
	Total	100.0	61.6	61.2	60.6	61.9
			(0.24)	(0.26)	(0.24)	(0.26)
Battered hake	Moisture	58.2	42.1	41.3	41.5	42.0
			(0.93)	(0.21)	(0.26)	(0.58)	(0.53)
	Lipids	6.6	13.5	14.2	14.1	13.0
		(0.70)	(0.45)	(0.43)	(0.52)	(0.36)
	Dry, lipid-free matter	35.2	35.7	35.1	35.6	35.3
	Total	100.0	91.3	90.6	91.2	90.3
			(0.42)	(0.56)	(0.57)	(0.76)

[a] Means of six determinations.
Numbers within brackets are standard errors ($S_{\bar{x}}$).

Table 5—Total polar compounds in frying oils and in food lipids (wt% on fat basis)

			Potatoes		Battered hake	
		Before frying	1st frying	6th frying	1st frying	6th frying
Unheated olive oil	frying oil	4.7	7.9	9.5	8.3	11.7
	food lipids	[a]	7.4	9.7	8.5	12.9
Used olive oil	frying oil	18.7	20.3	20.4	19.9	19.9
	food lipids	[a]	20.2	20.1	20.9	20.7
Unheated sunflower oil	frying oil	4.8	7.9	10.1	9.8	12.6
	food lipids	[a]	8.1	10.6	8.9	11.3
Used sunflower oil	frying oil	23.4	24.3	27.5	24.5	28.1
	food lipids	[a]	24.0	28.5	23.9	26.6

[a] Total polar compounds in food lipids before frying: Potatoes: 19.5% Battered hake: 14.1%.

the initial fresh oils (≈5%), diglycerides were the major compounds in the olive oil (Table 1) while the oxidized triglyceride monomers stand out in the sunflower oil. After use in domestic frying, a substantial increase was observed in the amounts of polymerization and oxidation compounds. Also a higher ratio of polymers/oxidized triglycerides occurred for the sunflower oil due to its high content in polyunsaturated fatty acids.

Table 2 shows quantitation of the major fatty acids in the initial oils and in the lipids extracted from the foods after first frying. Fatty acid composition was determined routinely in our laboratory, reproducibility being checked weekly. The standard deviation for the different fatty acids was below 0.31 for 6 determinations and hence the coefficient of variation was less than 5% for those fatty acids with concentrations higher than 6.2%. From Table 2, the food lipids were very similar to those of the initial frying oils, although the influence of the fat of the prefried product was still notable. This was clearly observed in all fatty acids where differences between food lipids and frying oils were greater than 30%. These corresponded to palmitic and oleic acids for the potatoes fried with olive oil, palmitic and linoleic acids for potatoes fried with sunflower oil, and oleic and linoleic acids for fish fried with olive oil.

Given the different fatty acid composition of the perfried food lipids and of the oils used in frying, the amount of fat absorbed from the fryer by the fried food could be calculated mathematically from the following expression:

$$100 \times \text{wt\% of } C_x \text{ in fried fat} = a \times \text{wt\% of } C_x \text{ in frying oil} + (100 - a)\text{wt\% of } C_x \text{ in prefried food fat} \quad (1)$$

in which

a = wt% of frying oil in fried food fat
C_x = fatty acid

Table 3 shows concentrations of frying oil in fried foods, calculated from the fatty acids where differences between food lipids and frying oils were greater than 30%. This calculation cannot be applied to the combination battered hake / sunflower oil as sunflower oil was also used in industrial prefrying. Consequently, there was no difference between fatty acid compositions of the two oils involved and reproducible results could not be expected.

Irrespective of the frying oil used and of food subjected to frying, more than 90% of the fried product fat came from the frying oil. Note the precision and reproducibility of the method used, as practically identical results were obtained in independent calculations from two fatty acids in each case. The results were very different from those reported in the frying of fresh fatty foods (Gall et al., 1983; Mai et al., 1978) where the final fat of the food seemed to have a very high proportion of the initial fat. This could have been due to the greater difficulty for fat interchange when the fat was not present in the surface layers of the food. Thus, results (Table 3) showed that the quality of the fried food depended basically on the oil used in its final preparation. However, there was no indication of the amount of initial food lipids retained by the fried food. Similar

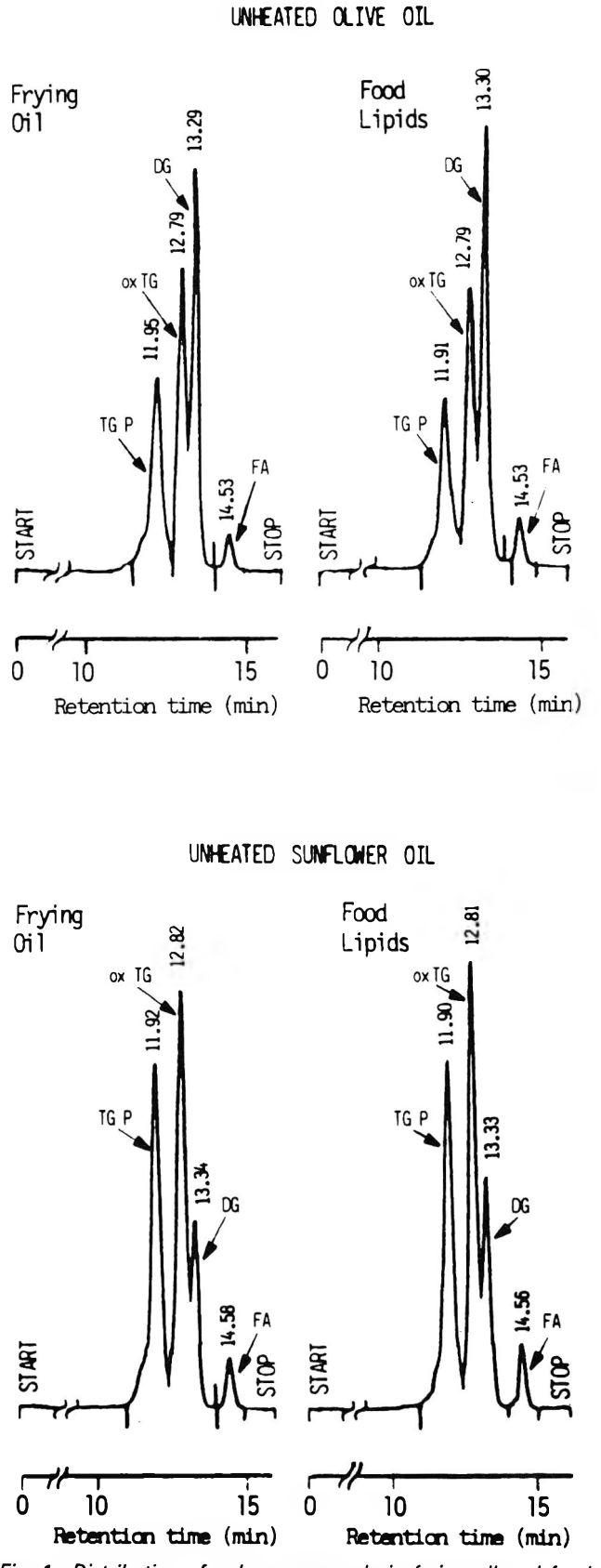

Fig. 1—Distribution of polar compounds in frying oil and food lipids from fried potatoes after the last frying, as determined by high performance size exclusion chromatography. Abbreviations: TG P - Triglyceride polymers, ox TG -Oxidized triglyceride monomers, DG -Diglycerides, FA - Fatty acids.

Table 6—Distribution of polar compounds in frying oils and food lipids after frying of prefried potatoes (mg/g)[a]

		After 1st frying				After 6th frying			
		TG P	ox TG	DG	FA	TG P	ox TG	DG	FA
Unheated olive oil	frying oil	13.8	19.6	40.8	4.7	24.8	28.4	38.1	3.7
	food lipids	12.2	20.4	36.3	5.1	27.2	30.6	35.2	4.0
Used olive oil	frying oil	70.4	73.5	51.4	7.7	79.5	70.8	47.8	5.9
	food lipids	73.2	76.1	46.8	5.9	71.5	73.6	49.4	6.4
Unheated sunflower oil	frying oil	22.9	32.9	17.4	5.7	38.8	39.3	17.2	5.7
	food lipids	23.4	29.3	19.1	9.2	41.4	42.5	18.6	4.5
Used sunflower oil	frying oil	131.6	78.3	25.2	7.9	144.3	95.6	26.8	8.3
	food lipids	126.0	83.6	23.4	7.1	150.5	98.4	29.6	6.5

[a] Abbreviations: TG P - Triglyceride polymers; ox TG - Oxidized triglyceride monomers; DG- Diglycerides; FA - Fatty acids.

Table 7—Distribution of polar compounds in frying oils and food lipids after frying of prefried battered hake (mg/g)[a]

		After 1st frying				After 6th frying			
		TG P	ox TG	DG	FA	TG P	ox TG	DG	FA
Unheated olive oil	frying oil	17.5	24.3	34.1	7.1	38.3	32.9	39.8	6.1
	food lipids	16.6	29.6	30.4	8.4	41.0	44.8	36.8	6.4
Used olive oil	frying oil	83.2	63.7	42.8	9.4	75.3	70.1	45.3	8.3
	food lipids	78.9	70.4	47.2	12.5	77.2	76.4	46.1	6.8
Unheated sunflower oil	frying oil	33.0	35.5	18.8	10.1	57.8	46.9	14.4	7.0
	food lipids	34.8	31.5	14.3	8.4	50.4	40.1	16.2	6.3
Used sunflower oil	frying oil	125.3	83.9	26.1	9.7	146.5	94.1	31.2	9.2
	food lipids	132.1	76.0	22.4	8.5	150.0	84.4	24.6	6.7

[a] Abbreviations: TG P - Triglyceride polymers; ox TG - Oxidized triglyceride monomers; DG- Diglycerides; FA - Fatty acids.

information on the amount of food lipids that have passed to the fryer would be interesting. However, the amount of food fat was not enough to significantly modify the fatty acid composition of the frying oil. The fatty acid compositions of the oils after the first frying were not included as they were practically identical to the initial oils.

Contamination of the fryer oil by the food could, however, be determined from the amount of fat present in the prefried and fried foods. Table 4 shows the quantitative changes in the major components of the food. The results for fried foods were expressed on the food weight obtained from 100 g of prefried food. Thus, from the values shown in Table 4, both the loss of moisture and the net gain of lipids can be directly determined by subtraction. As can be seen, the changes that occurred depended clearly on the food subjected to frying, as the final process of frying was the same for both products. The battered hake, with lower surface/volume and surface/weight ratios, had a lower loss of total weight and water weight. However, surprisingly, the amount of fat absorbed was much higher than that retained by the potato. On the other hand, dry, lipid-free matter remained constant in the two products.

In order to determine whether significant differences ($P \leq 0.05$) occurred between mean values of fried food fat due to type of oil, the level of altered fatty acids and the type of food, *t*-tests were applied. The test was based upon 24 pairs of data in the case of the type of food and highly significant differences ($P < 0.01$) were found between potatoes and fish. For the type of oil and the level of altered fatty acids the tests were based upon 12 pairs of data and no significant differences were found either for potatoes or for fish. Taking into account the amount of food fat derived from the frying oil (≈92.0%) and the mean quantities of fat in both foods (4.6 and 6.6% in prefried foods and 8.5 and 13.7% in fried foods), we could determine that 85% and 83% of prefried food lipids had been released into the frying oil. Thus, not only did the frying oil influence the nature of the product, but at the same time the food lipids gradually modified the nature of the frying oil.

Table 5 shows the results obtained from quantitation of the polar altered fatty acid compounds after the first and last frying. As can be seen, both the samples of lipids extracted from the fried foods and the frying oils have been evaluated to analyze the possibility of a preferential adsorption of polar compounds on the surface of the product. The results indicated clearly that the amounts of altered compounds were higher when an unheated oil was used in the final preparation of the food. However, no appreciable differences were observed between the food lipids and the frying oils.

A more thorough analysis of the differences in altered compounds could be carried out comparing the distributions of polar compounds. Fig. 1 shows the efficiency of the separation obtained by means of HPSEC where no differences were observed between frying oils and lipids from potatoes after the last frying. Nevertheless, distribution of minor glyceride components depended on the frying oil, as polymers and oxidized triglycerides were higher when sunflower oil was used in the final preparation of the foods.

Note that differences in distribution of polar compounds could be associated with differences in nutritional significance of the fats. While diglycerides and fatty acids are the usual compounds originating during fat metabolism, polymers and oxidized triglycerides contain modified acyl groups which would impair the nutritional value of the fats. Thus, among the oils we used, foods fried with olive oil are better from a nutritional standpoint.

Quantitative results are given in Table 6 and 7, for potatoes and battered hake, respectively. As evident from comparison with initial oils (Table 1), polymers and oxidized triglycerides were the major compounds which originated during frying, which demonstrates the importance of temperature and oxygen. Changes in the amounts of diglycerides were lower, regardless of the high moisture content of the foods. On the other hand, the results indicated that there was no preferential adsorption during the final preparation of prefried foods for any of the most significant altered fat compounds. As preferential adsorption of fatty acids and oxidized products in the external layers of certain fresh foods during frying has been reported (Pokorny, 1980), our results could be due to the nature of the foods fried. Possibly the adsorption of polar compounds occurred during the prefrying phase, with the surface being inactivated afterwards, during final preparation of the food.

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—*Continued on page 1650*

Interactions Among Oil Components During Adsorption: Effects on Carotenoids and Peroxides

JORGE F. TORO-VAZQUEZ

ABSTRACT

Soybean, cottonseed, corn and squash seed oils were treated for 3 h with activated carbon (1%, 2.5%, and 5%) in a batch adsorption system at 50 and 90°C. Peroxides (P_a) and carotenoids (C_a) were determined after adsorption. Equations that described the P_a and C_a variability were developed through discriminatory multiple regression analysis using as independent variables chemical characteristics of the oils (iodine and saponification index, peroxides, carotenoids and free fatty acids, saturated/unsaturated fatty acids ratio), process temperature and adsorbent concentration. Variables in the models explained ≈85% and ≈93% of the total variability observed in the P_a and C_a concentrations respectively, independent of oil source.

INTRODUCTION

LIPID AUTOXIDATION and the products of peroxides decomposition are among the contaminants which most greatly influence oil/fat quality and stability (Paik et al., 1976; Yoshioka et al., 1974; Yoshida and Kaneda, 1974). Current refining technology partially eliminates products of oxidation (Fedeli, 1987a, 1987b). Autoxidation of fat/oil proceeds via typical free radical mechanisms (Cash et al. 1988; Nawar, 1985). The reactions involved are affected by several factors including fatty acid composition (Nawar, 1985; Miyashita and Takagi, 1986), presence of pro-oxidants (Miyashita and Takagi, 1986; Yanishlieva and Kortenska, 1989) or antioxidants (Warner and Frankel, 1987; Lai et al., 1989). Warner and Frankel (1987) have shown that stability of refined soybean oil exposed to light was increased by the addition of 1 to 20 ppm of β-carotene. The β-carotene has been regarded as a singlet oxygen quencher (Nawar, 1985). This quenching effect seemed to depend on the presence of tocopherols, which protect β-carotene from free radical autoxidation (Warner and Frankel, 1987; Niki, 1987). Miyashita and Takagi (1986) determined the induction periods for autoxidation of methyl esters as 1860 h for methyl oleate, 91 h for methyl linoleate, and 34 h for methyl linolenate. The same authors, reported that free fatty acids accelerated peroxide decomposition in model systems (Miyashita and Takagi, 1986).

The process of bleaching, mainly designed for pigment removal (Taylor et al., 1989), has also had effects on the removal of trace metals, phospholipids (Brown and Snyder, 1989), peroxides (Nkpa et al., 1989; Toro-Vazquez, 1990; Toro-Vazquez et al., 1990) as well as products of peroxide decomposition (Boki et al., 1989, 1990). Bleaching is the only step in the refining of vegetable oils that uses adsorption. Although acid-activated montmorillonite is the standard bleaching clay used by the food oils industry, the reasons for its dominance over other adsorbents is not clear (Taylor et al., 1989). There is interest in examining other materials as alternatives to conventional bleaching clays (Nkpa et al., 1989; Palaniappan and Proctor, 1990; Proctor and Palaniappan, 1989; Taylor et al., 1989). Activated carbon is one of the most widely used adsorbents because of its low price, and wide spectrum of adsorption capability, unmatched by other materials (Weber, 1985). However, there is little information regarding use and adsorption behavior of activated carbon for vegetable oils.

The process of adsorption involves separation of a substance (i.e., adsorbate) from one phase accompanied by its accumulation or concentration on the surface of the adsorbing phase. A quantitative equilibrium between the adsorbate concentration in each phase is defined for each combination of specific adsorbate, adsorbent, and conditions of temperature, pH, and ionic strength (Weber, 1985). Brown and Snyder (1989) and Toro-Vazquez (1990) have pointed out that the study of selective adsorption of unwanted components from unrefined oils could lead to increased efficiency of refining methods. Better understanding of bleaching procedures, as well as better control of physicochemical/functional properties of refined oils could also result. Our objective was to study the effects of some chemical components of unrefined oils on peroxides and carotenoid concentrations during an adsorption process with activated carbon.

MATERIALS & METHODS

Adsorption experiments

Soybean, corn, and cottonseed unrefined oils were obtained from local processors. Two batches of squash (*Cucurbita moschata*) seed oil were extracted following the procedure described by Toro-Vazquez et al. (1990). Briefly, a mixture of dry milled seed and n-hexane (ratio 1:4) was magnetically stirred for 30 min, filtered (Whatman paper #2) and the solvent evaporated (60°C under vacuum) from the filtrate.

The oils were distributed in aliquots (200 mL) in amber bottles, and stored under nitrogen atmosphere and refrigeration (4°C). The unrefined oils were analyzed for iodine index (II_u), saponification value (SV_u), percentage of free fatty acids (FFA_u, as oleic acid) and peroxide value (P_u) (AOAC, 1980). The ratio of unsaturated to saturated fatty acids (UFA/SFA) of each unrefined oil was calculated from reported data (Swern, 1982; Toro-Vazquez, 1990). Carotenoids (C_u) were determined by absorbance at 417 nm of an oil solution in hexane (≈2.5% w/v); the Zscheile et al. (1944) equation was used:

$$\frac{\text{mg carotenes}}{\text{kg of oil}} = \frac{(\text{absorbance at 417 nm})\,(\text{sample volume, mL})}{(0.204)\,(\text{sample weight, g})}$$

To eliminate secondary effects such as particle size, moisture content, etc, the same lot of activated carbon (Clarificantes Mexicanos; Santa Clara, Estado de México, Mex.) was used in all experiments after sieving (U.S. standard, −14/+24), washing (mixing for 24 hr in 2 volumes of deionized water), filtering and drying (100°C for 24 hr). Factorial combinations of process temperature (50 and 90°C) and activated carbon (AC) concentration (1, 2.5, and 5% w/w) were distributed among the oil bottles (soybean, corn, cottonseed and squash seed) in a complete randomized experimental design with 2 replicates. Adsorption was done under vacuum in a rotary evaporator (Büchi RE 110; Büchi Laboratoriums-Technik AG, Flawil, Switzerland); Proportions of oil and adsorbent were weighed in the evaporator flask (1L), to obtain 200g final weight. After 3 hr constant stirring, the mixture was centrifuged (5000 rpm/15 min), filtered and the oil analyzed for peroxides (P_a) and carotenoids (C_a).

Statistical analysis

Multiple regression analysis was used to develop equations that described the variability in P_a and C_a, using as independent variables

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Table 1—Descriptive statistics of the vegetable oils chemical properties

	n[a]	Mean	Std dev	Minimum	Maximum
Unrefined Oils					
Peroxide value (mEq/kg oil)	5	18.09	22.11	2.04	56.24
Carotenoids (mg/kg oil)	5	68.67	51.10	16.13	128.01
Free fatty acids (% as oleic acid)	5	0.99	1.04	0.17	2.79
II/SV[b]	5	0.46	0.15	0.37	0.73
SFA/UFA[c]	4	0.24	0.09	0.17	0.36
Adsorbed oils					
Peroxide value (mEq/kg oil)	53	11.01	8.57	0.20	38.05
Carotenoids (mg/kg oil)	53	37.94	35.17	4.67	107.99

[a] Number of replicates; each replicate was done in duplicate.

[b] Ratio iodine index to saponification value.

[c] Ratio of saturated fatty acids concentration to unsaturated fatty acids concentration.

the chemical characteristics of the unrefined oils, the process temperature (T°), the AC concentration, their quadratic effects and linear interactions. A regression model is a separation of each observation into two parts. One is the predictable portion, which can be assigned to sources (i.e., explanatory variables) known to affect the response variable. The other, the residual portion, is assumed to be due to random variability. The variable selection for regression modeling was made under the basis of residual analysis, as well as the magnitude of the "variance constant" (Vc) for the estimated β-regression coefficients of explanatory variables in the model (Allen and Cady, 1982). The standard error of the estimated β-regression coefficients, is given by

$$\text{Standard error of } \beta_i = [(\text{MSRes})\ (Vc_i)]^{1/2}$$

where β_i is the regression coefficient for the independent (or explanatory) variable X_i, MSRes is the mean square of the residuals in the model and $Vc_i = 1/\Sigma(X_{ij} - \hat{X}_{ij})$, where "j" = the different values of X_i, and $\hat{X}_{ij}$ represents the magnitude of X_{ij} that can be predicted by other independent variables previously fitted into the model. Therefore, a good criterion to select a reduced model (i.e., the model with the least number of variables that provided an acceptable determination coefficient, $R^2 > 0.80$), is to evaluate the magnitude of the MSRes and the Vc_i in the reduced model with respect to their values in the general model (e.g., model with higher number of variables than the reduced model). If the Vc_i for the regression coefficients decrease without significant change in the MSRes, the effect might be a decrease in standard error of the β estimates, and therefore in the standard error of the prediction. Additionally, the smaller the Vc of the variables fitted in the model the higher the independence (i.e., noncorrelation) among explanatory variables.

RESULTS & DISCUSSION

OIL COMPOSITION data were analyzed independently of source, i.e., the data were considered as all from the same source. This assumption generalized the inference obtained from statistical analysis, because the application of the regression models obtained were not restricted by the oil source. The descriptive statistics of the oils chemical properties are shown in Table 1. The reduced models that best described the variability in P_a and C_a are shown in Table 2. Several reduced models were developed essentially as well as those shown; however, the models in Table 2 were those with the greatest R^2, and the lowest Vc_i and standard deviations.

In the models evaluated, the T° effect did not explain a meaningful amount of the P_a and C_a variability. Using squash seed oil, Toro-Vazquez (1990) observed no significant difference between the 50 and 90°C peroxides and carotenoids adsorption isotherms with activated carbon. With peroxides, the regression model indicated the P_a was mainly a function of the FFA_u, as well as of linear interactions among the P_u, the AC, the C_u and the II_u/SV_u. The sign of the β coefficients indicates whether the respective variable (or interaction) had a decreasing (−) or increasing (+) effect on P_a (or C_a) (Table 2). The

Table 2—Regression models that described the variability in peroxides (P_a) and carotenoids (C_a) concentration in vegetable oils (soybean, cottonseed, corn, and squash seed) after an adsorption treatment (3 hr at 50 and 90°C) with activated carbon at concentrations of 1, and 2.5, and 5% w/w

Dependent variable	Variable description[a,b]	Coefficient (β)	Std error[c]	p[d] (β = 0)	R^2 of model
P_a	Constant	3.5810	0.9409	0.0004	0.8495
(F = 816.8;	P_u	0.8519	0.2329	0.0006	
s.d.[e] = 2.34;	P_u·AC	−0.0851	0.0135	0.0000	
n = 53)	P_u·C_u	−0.0087	0.0015	0.0000	
	P_u·II_u/SV_u	1.6419	0.5827	0.0071	
	FFA_u	−3.0160	0.8342	0.0007	
C_a	Constant	10.4900	1.8587	0.0000	0.9323
F = 1564;	C_u^2	0.0063	0.0003	0.0000	
s.d. = 9.33;	AC·C_u	−0.0661	0.0095	0.0000	
n = 53)					

[a] Variance constant of the independent variables in the models < 0.13.

[b] P_u, peroxides value (mEq/kg of oil) in unrefined oils; AC, activated carbon (% w/w); C_u, carotenoids in unrefined oils (mg/kg oil); II_u/SV_u, iodine index/saponification value in unrefined oils; FFA_u, percentage of free fatty acids (as oleic) in unrefined oils.

[c] Standard error associated with each of the β coefficients.

[d] Probability that the β coefficients equal zero.

[e] Standard deviation of the model.

variables included in the model explained ≈85% (R^2 = 0.8495) of the total variability observed in P_a; this indicated that additional variables should be considered in order to develop predictive models.

Previous results (Toro-Vazquez, 1990; Toro-Vazquez et al., 1990), have suggested that the peroxides behavior during the adsorption process might be the result of a balance between peroxide formation (Nawar, 1985), decomposition (Nawar, 1985; Nkpa et al., 1989; Pardun et al., 1968), and adsorption (Boki et al., 1989; Wiedermann, 1981). The significance of the P_u and $P_u \cdot II_u/SV_u$ effects, as well as the sign of their respective β coefficients, might point out the importance of peroxide formation during the adsorption process. This could be through the effects of peroxides concentration, type and degree of fatty acid unsaturation (measured indirectly as the ratio II_u/SV_u) on reaction rates of the initiation and propagation autocatalytic reactions. The interaction $P_u \cdot C_u$ and the sign of the respective β coefficient, indicated that as the oil carotenoids concentrations were lower the oils P_a were higher. This seemed to indicate the significance of the carotenoids antioxidant activity (Warner and Frankel, 1987) during the adsorption process, probably through limiting the occurrence of initiation reactions.

The importance of a decreasing linear effect of the FFA_u in the P_a model, was in agreement with the FFA pro-oxidant activity (Miyashita and Takagi, 1986). Presumably, the FFA catalyzed the peroxides homolytic cleavage (Miyashita and Takagi, 1986). Finally, the importance of the peroxides adsorption factor was accounted for in the model through the presence of the $P_u \cdot AC$ interaction. Boki et al. (1989) have shown that the original peroxides concentration (i.e., P_u), as well as the adsorbent concentration (i.e., AC) determine peroxide adsorption from vegetable oils.

In contrast with the P_a, the C_a behavior was a function of a quadratic effect of the C_u and a linear interaction between the C_u and the AC concentration (Table 2). The model explained ≈93% (R^2 = 0.9323) of the total variability observed in C_a. The positive sign of the β coefficient for the C_u^2 effect, indicated that carotenoids decreased in a quadratic relation toward adsorption equilibrium. The amount of carotenoids adsorbed was a function of the original carotenoids concentration (i.e., C_u) and the adsorbent concentration (i.e., AC) (Table 2).

CONCLUSIONS

FROM THE VARIABLES and interactions in the models developed we concluded that, the decrease in carotenoid concentration in oils was mainly due to the adsorption. In contrast,

the peroxides concentration after adsorption, was described by a balance among peroxide formation, decomposition, and adsorption. Predominance of any of these depends upon the interaction among several variables, mainly, the adsorbent concentration (i.e., AC), stage of oxidation (i.e., P_u), susceptibility of the fatty acids to react with oxygen (i.e., II_u/SV_u), and the presence of compounds with antioxidant (i.e., C_u) or pro-oxidant (i.e., FFA_u) activity. The study of factors that determine selective adsorption, rates of adsorption equilibrium and chemical reactions in the process will promote use of adsorption in oil refining.

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Ascorbyl Palmitate Efficacy in Enhancing the Accelerated Storage Stability of Canola Oil

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ABSTRACT

The effect of various levels of ascorbyl palmitate (AP) and of butylated hydroxyanisole/toluene (BHA/BHT) on the accelerated storage stability of canola oils was determined by sensory, gas liquid chromatographic (GLC) and chemical evaluations. In Schaal oven tests (65°C, 0–16 days), chemical, GLC and trained sensory panel data indicated that 200 ppm AP retarded autoxidation in stored canola oils. Monoglyceride citrate (MGC) addition to oils containing 200 ppm AP did not enhance oil stability. Fluorescent light tests (7500 lux, 24°C, 0–24 hr) showed that 200 ppm AP, with or without MGC, had a limited effect in protecting canola oil from photooxidation. BHA/BHT, at 100 ppm each, with MGC, did not improve canola oil stability.

Key Words: canola-oil, storage-stability, ascorbyl-palmitate, sensory, antioxidants

INTRODUCTION

CANOLA OIL, with its relatively high proportion of polyunsaturated fatty acids has the potential to develop undesirable odors and flavors during storage. The primary and secondary oxidation products of polyunsaturated fatty acids have been implicated as the major cause of unpleasant flavors in stored oils (Dupuy et al., 1977; Jackson and Giacherio, 1977; Waltking and Goetz, 1983; Frankel et al., 1984; Stevenson et al., 1984; Vaisey-Genser and Eskin, 1987).

Reported research is limited on the efficacy of antioxidants in improving the oxidative stability of canola oil. The antioxidants studied have either proven to be ineffective, as with a combination of BHA/BHT and MGC (Vaisey-Genser and Ylimaki, 1985; Hawrysh et al., 1988), or were not licensed for use in Canada. Although both tertiary butylhydroquinone and anoxomer were effective in extending canola oil storage stability, they cannot be added to vegetable oils produced for sale in Canada.

Reports have suggested that addition of ascorbyl palmitate (AP) to oils (such as soybean, corn and peanut) is effective in promoting autoxidative stability during storage (Cort, 1974). In comparing the effectiveness of 2000 ppm AP, 200 ppm BHA and 200 ppm propyl gallate in stored (28°C) sunflower and rapeseed oils, Sedlacek (1968) concluded that AP was the best for oil stabilization. Similar conclusions were also made by Ahmad et al. (1983). AP may offer advantages over other antioxidants in terms of efficacy, safety and positive labelling connotations. In addition, AP is licensed for use as a food additive in Canada. However, evaluations of the effectiveness of AP in retarding autoxidative changes in canola oil are lacking.

Our objective was to evaluate the efficacy of varying levels of AP and of a combination of BHA and BHT in retarding oxidation of canola oils subjected to accelerated storage conditions, specifically the Schaal oven (at 65°C) and fluorescent light tests.

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MATERIALS & METHODS

FULLY REFINED, bleached and deodorized canola oil, containing no antioxidants or citric acid, was obtained from Canbra Foods (Lethbridge, Alberta). AP was provided by Hoffman-LaRoche (Etobicoke Ontario); BHA, BHT, and MGC were obtained from Griffiths Laboratory (Toronto, Ontario).

The quality of the fresh canola oil was confirmed via analyses for fatty acid composition (Bannon et al., 1982) and iodine (IUPAC, 1987) and peroxide values (PV) (AOCS, 1979). Three separate lots of oil were obtained to represent three replicates. The following treatments (T) were prepared for each of the three replicates: T1, canola oil with no antioxidant (control); T2, canola oil with BHA/BHT (100 ppm of each) and MGC (containing 50 ppm citric acid); T3, canola oil with AP (100 ppm); T4, canola oil with AP (200 ppm); T5, canola oil with AP (200 ppm) and MGC (50 ppm citric acid); T6, canola oil with no antioxidant - frozen (−30°C) at the time of treatment preparation. Samples of T6 were used as blind controls and bland references for sensory evaluations.

For each treatment within each replicate, the oil was heated to 80°C in the presence of air and the antioxidant was incorporated (BHA, BHT, MGC were added neat as per industry practice). Due to its low solubility in canola oil, it was necessary to incorporate AP as a 5% solution in ethanol. Each oil treatment was stirred for 30 min at 80°C, and for an additional 30 min while cooling. The oil was placed into amber glass bottles (500 mL), flushed with nitrogen and held overnight at 5°C for use the following day. For each replicate, sufficient oil for each treatment was prepared for use in both storage tests. Procedures for the Schaal oven and fluorescent light tests were similar to those previously reported (Hawrysh et al., 1988) with the following changes. Oil samples for the Schaal oven test were stored for 0, 4, 8, 12 and 16 days. For the fluorescent light test, samples were removed for analysis after 0, 8, 16 and 24 hr of illumination. After each storage period, oil samples were divided into 3 200 mL glass vials, flushed with nitrogen and frozen (−30°C) for later sensory, chemical, and gas liquid chromatographic (GLC) analyses (Schaal oven samples only). Prior to analyses, samples were kept in a dark area while thawed in water (23°C) for 5 min.

Sensory evaluations were conducted by seven trained panelists who had been screened on the basis of 12 triangle tests. Selected panel members were intensively trained for 7 wks according to the procedures of Cross et al. (1978). During training, judges learned to quantify overall odor and flavor intensities of a range of prepared canola oil samples which were similar in quality to those expected during the experiments. They also identified and quantified the strength of specific odor and flavor notes such as buttery, fishy, grassy, nutty, painty, rancid, and sweet in the prepared oil samples.

During the experimental period, panelists evaluated the overall odor and flavor intensity of six randomly presented oil samples (T1-T6) and two reference samples (identical to T6, the hidden control) using the AOCS 10-point intensity scale (10 = bland, 1 = extremely intense, Warner, 1985). Odor and flavor intensity values (OIV and FIV, respectively) for individual notes were obtained using a 6-point scale (0 = none, 5 = very strong). Details of procedures for sensory analysis appear in Hawrysh et al. (1988). For each replicate, panelists evaluated Schaal oven samples stored for 0, 8, 12 and 16 days and all samples from the fluorescent light test. All panel evaluations were conducted in an atmospherically controlled sensory panel room equipped with individual booths and red fluorescent lighting.

Chemical analyses of the oils included determinations of PV, thiobarbituric acid (TBA) values at 452 nm (Fioriti et al., 1974), and UV absorbance ($E^{1\%}_{1cm}$) at 234 nm using the IUPAC method (IUPAC, 1987). Off-flavor volatiles in oil samples subjected to Schaal oven storage were determined by GLC using the method described by Tokarska et al. (1986). Data for sensory evaluation and chemical analyses were subjected to analyses of variance according to a strip-plot

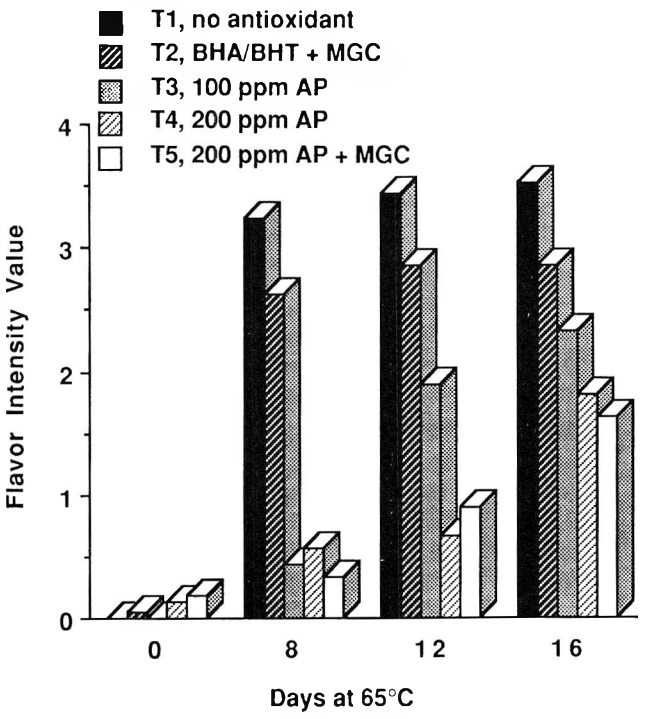

Fig. 1—Effect of antioxidants on painty flavor development in canola oils stored at 65°C.

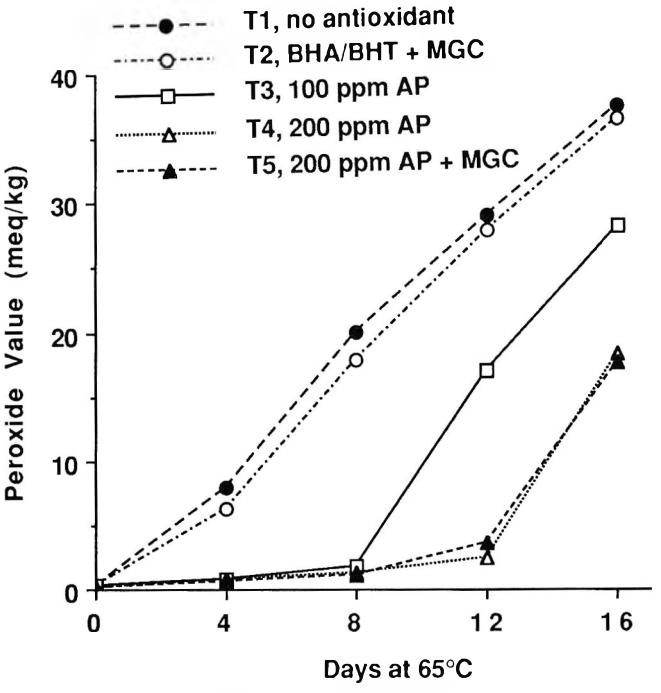

Fig. 2—Effect of antioxidants on peroxide development in canola oils stored at 65°C.

Table 1—Fatty acid composition of canola oil upon receipt

Fatty acid		Percentage of Total
Palmitic acid	(C16:0)	3.6
Palmitoleic acid	(C16:1)	0.2
Stearic acid	(C18:0)	1.6
Oleic acid	(C18:1)	59.3
Linoleic acid	(C18:2)	20.3
Linolenic acid	(C18:3)	10.6
Arachidic acid	(C20:0)	0.6
Gadoleic acid	(C20:1)	1.7
Behenic acid	(C22:0)	0.3
Erucic acid	(C22:1)	0.8

experimental design as outlined by Milliken and Johnson (1984). Where appropriate Student-Newman Keul's Multiple Range test was used to identify significant differences among treatment means.

RESULTS & DISCUSSION

THE CANOLA OIL was similar in initial fatty acid composition (Table 1) to that reported for canola oil by Vaisey-Genser and Ylimaki (1985) and Hawrysh et al. (1988). The PV of the fresh oil was 0.21 and the iodine value was 119.

Schaal oven test

Data for the sensory analysis of canola oils subjected to Schaal oven storage (Table 2) showed that AP protected the canola oil from oxidation during the accelerated storage test. In comparison, T2 (BHA/BHT + MGC treated oil) was ineffective in inhibiting canola oil degradation. After 8 days of storage, T1 and T2 were described as mild to moderately intense in odor and flavor while T6 (the hidden control) was bland. The odor and flavor intensity of the AP treated samples (T3, T4 and T5) were also scored as relatively bland. At 12 days, the odor scores for the samples treated with 100 ppm AP (T3) declined, while those for oils treated with 200 ppm AP (T4 and T5) remained high. At 16 days odor scores for T4 and T5 decreased but were higher ($P < 0.05$) than T1 and T2. At 12 and 16 days storage, flavor scores for the oil samples showed trends similar to those described for odor scores. Although the flavor scores for T4 and T5 declined gradually throughout storage, they remained significantly better than those for T1 and T2.

To further characterize each of the canola oil samples, a sensory profile was obtained consisting of odor and flavor descriptions (notes) and note intensities (intensity values). The predominant odor and flavor description given to stored oils was painty. The presence of painty odor and flavor notes was indicative of the severe deterioration in oil quality that occurred during Schaal oven storage (Cowan et al., 1970; Mounts et al, 1978). The development of painty flavors (Fig. 1) clearly showed the effectiveness of AP in promoting canola oil stability. After 8 days, painty flavor intensity values (FIV) for T1 and T2 were moderately intense (scores of about 3 on the 6-point scale). At 12 days, the painty FIV for T3 (100 ppm AP) increased to slightly intense, while those for T4 and T5 (200 ppm AP) remained relatively low. The production of painty odor followed identical trends to those described for painty flavor development.

Results for chemical analyses (Fig. 2 and Table 3) also indicated that AP delayed oxidation of canola oils. Peroxide development (Fig. 2) during storage was not affected by BHA/BHT + MGC but was markedly retarded by AP. Vaisey-Genser and Ylimaki (1985) and Hawrysh et al. (1988) also concluded that BHA/BHT was ineffective in promoting canola oil stability. Peroxide values indicate that both levels of AP were beneficial in delaying oxidation. The induction period for peroxide development was extended to 8 and 12 days by the presence of 100 or 200 ppm AP (with or without MGC), respectively. After storage of up to 12 days, the oil samples stabilized with 200 ppm AP (T4 and T5) had PV in the range of 2.4 to 3.6 meq/kg. Researchers (Vaisey-Genser and Ylimaki, 1985) have concluded that canola oils with PV of 24 meq/kg were still of good sensory quality. In our present study the oils treated with 200 ppm AP and stored for 12 days could still be considered of good quality.

Data for TBA values for canola oils exposed to the Schaal oven test (Table 3) showed that during storage T1 (the untreated control) and T2 (BHA/BHT + MGC treated) developed

Table 2—Odor and flavor intensity scores for canola oil, with and without antioxidant, following accelerated storage at 65°C. Means and standard errors

Characteristic	Storage time (days)	Canola oil treatment: Untreated control (T1)	BHA/BHT (100 ppm ea) + MGC (T2)	AP (100 ppm) (T3)	AP (200 ppm) (T4)	AP (200 ppm) + MGC (T5)	Blind control (T6)	SEM[f]
Overall odor intensity[g]	0	9.8	9.7	9.6	9.5	9.4	9.8	0.24
	8	5.1[c]	5.6[c]	8.7[ab]	9.0[ab]	8.1[b]	9.5[a]	0.24***
	12	4.5[e]	4.7[e]	6.9[d]	9.0[b]	8.2[c]	9.8[a]	0.20***
	16	3.5[d]	4.6[c]	5.0[c]	6.9[b]	6.8[b]	9.8[a]	0.33***
Overall flavor intensity[g]	0	9.8	9.7	9.5	9.4	9.4	9.8	0.10
	8	4.2[c]	4.9[c]	8.0[b]	8.1[b]	7.5[b]	9.5[a]	0.27***
	12	3.8[e]	4.7[d]	6.3[c]	7.6[b]	6.8[bc]	9.5[a]	0.26***
	16	3.5[d]	4.4[cd]	5.5[bc]	6.7[b]	6.6[b]	9.7[a]	0.40***

[a-e] Means within the same row sharing a common letter are not significantly different at P≥0.05.
[f] Standard error of the mean.
[g] Ten point intensity scale where 10 = bland, 1 = extremely intense. Means are averages of 21 scores (7 panelists, 3 replications).
*,**,*** Significant differences among canola oil treatments at P<0.05, P<0.01 and P<0.001, respectively.

Table 3—Chemical analyses of canola oil, with and without antioxidant, following accelerated storage at 65°C. Means[f] and standard errors

Characteristic	Storage time (days)	Canola oil treatment: Untreated control (T1)	BHA/BHT (100 ppm ea) + MGC (T2)	AP (100 ppm) (T3)	AP (200 ppm) (T4)	AP (200 ppm) + MGC (T5)	SEM[g]
TBA Value[h] (452 nm)	0	5.81[b]	6.00[b]	6.94[a]	7.09[a]	7.14[a]	0.23**
	4	12.43[a]	11.80[a]	7.50[b]	8.14[b]	7.90[b]	0.83**
	8	18.37[a]	17.38[a]	8.49[b]	8.36[b]	8.23[b]	0.89***
	12	18.14[a]	17.69[a]	14.97[a]	8.55[b]	9.02[b]	1.23**
	16	19.78[a]	20.01[a]	18.23[a]	16.19[b]	15.81[b]	0.44***
$E^{1\%}_{1cm}$ (234 nm)[i]	0	4.04	4.08	4.09	4.06	4.05	0.03
	4	4.70[a]	4.58[b]	4.19[c]	4.16[c]	4.13[c]	0.03***
	8	5.80[a]	5.67[a]	4.21[b]	4.18[b]	4.17[b]	0.05***
	12	6.81[a]	6.65[b]	5.52[c]	4.22[e]	4.42[d]	0.04***
	16	7.78[a]	7.72[a]	6.78[b]	5.84[c]	5.76[c]	0.08***

[abcde] Means within the same row sharing a common letter are not significantly different at P≥0.05.
[f] Means are averages of 6 determinations (2 per each of 3 replicates).
[g] Standard error of the mean.
[h] Thiobarbituric acid value.
[i] Not corrected for triglyceride absorption.
**, ***Significant differences among canola oil treatments at P<0.01 and P<0.001, respectively.

Table 4—Off-flavor volatiles in canola oil, with and without antioxidant, following accelerated storage at 65°C. Means[a]

Compounds[b]	Storage Time (days)	Canola oil treatment: Untreated Control (T1)	BHA/BHT (100 ppm ea) + MGC (T2)	AP (100 ppm) (T3)	AP (200 ppm) (T4)	AP (200 ppm) + MGC (T5)
Total volatiles	0	6.50	6.50	7.87	3.37	3.76
	4	9.36	9.47	1.71	2.92	2.75
	8	77.86	42.05	8.25	4.52	4.02
	12	120.40	84.56	58.97	9.19	10.02
	16	148.27	107.97	109.52	30.46	28.92
Butane/Pentane	0	0.12	0.12	0.15	0.20	0.08
	4	5.14	3.58	0.27	0.38	0.41
	8	48.36	20.97	1.49	1.81	1.28
	12	51.07	30.84	25.81	2.79	3.37
	16	62.26	43.80	46.27	12.81	9.76
2,4-decadienals	0	–	–	–	–	–
	4	0.35	0.38	–	–	–
	8	5.56	2.88	0.43	0.33	0.26
	12	19.95	12.93	12.68	0.78	0.97
	16	24.29	19.31	14.62	3.48	3.45

[a] Means are averages of 6 analyses (3 replicates, duplicate analyses).
[b] Determined as ppm from GLC analyses.

dienals (Jacobson et al., 1964) at a much faster rate than T3, T4, and T5 (the AP treated oils). Samples containing 100 ppm AP (T3) had marked increases in TBA values at 12 days. However, TBA values for the samples with 200 ppm AP (T4 and T5) remained low until 16 days. Increases in TBA values for BHA/BHT + MGC treated canola oils were also noted by Hawrysh et al. (1988). Results for absorbance ($E^{1\%}_{1cm}$) at 234 nm (Table 3) showed trends similar to those described for PV and TBA analyses. Since the absorbance ($E^{1\%}_{1cm}$) at 234 nm indicates the state of autoxidation of an oil (IUPAC, 1987), findings similar to those obtained for PV were expected.

GLC data (Table 4) supported the results of sensory and chemical analyses. Results for total volatiles, butane/pentane and 2,4-decadienals, which have been identified as major "off-flavor" volatiles in stored canola oils (Tokarska et al. 1986), showed that 200 ppm AP, with or without MGC, was effective in delaying formation of volatiles. At such storage time, oil samples treated with 200 ppm AP had substantially lower levels of volatiles than either the untreated control (T1) or the BHA/BHT + MGC treated (T2) canola oils.

Thus, data indicated that the addition of 200 ppm AP to canola oil was more effective than 100 ppm AP in promoting stability. Pongracz (1973) also reported that the incorporation of increasing amounts of AP into sunflower oils resulted in

Table 5—Odor and flavor intensity scores for canola oil, with and without antioxidant, following exposure to fluorescent light (7500 lux). Means[d] and standard errors

Characteristic	Storage time (hr)	Canola Oil Treatment: Untreated control (T1)	BHA/BHT (100 ppm ea) + MGC (T2)	AP (100 ppm) (T3)	AP (200 ppm) (T4)	AP (200 ppm) + MGC (T5)	Hidden control (T6)	SEM[d]
Overall odor intensity[e]	0	9.7	9.8	9.6	9.5	9.4	9.8	0.20
	8	7.3[b]	7.3[b]	7.8[b]	8.8[ab]	8.5[ab]	9.7[a]	0.34**
	16	5.9[b]	6.7[b]	6.9[b]	7.1[b]	7.0[b]	10.0[a]	0.38***
	24	5.7[c]	5.7[c]	6.4[c]	7.1[b]	7.3[b]	9.7[a]	0.22***
Overall flavor intensity[e]	0	9.6[abc]	9.8[ab]	9.5[bc]	9.4[c]	9.4[c]	9.8[a]	0.08*
	8	7.2[b]	7.1[b]	7.0[b]	7.0[b]	7.1[b]	10.0[a]	0.34***
	16	5.8[b]	6.1[b]	6.0[b]	6.2[b]	6.5[b]	10.0[a]	0.33***
	24	5.3[b]	5.0[b]	5.9[b]	6.0[b]	5.5[b]	9.7[a]	0.27***

[abc] Means within the same row sharing a common letter are not significantly different at P≥0.05.
[d] Standard error of the mean.
[e] Ten point intensity scale where 10 = bland, 1 = extremely intense. Means are averages of 21 scores (7 panelists, 3 replications).
*,**,*** Significant differences among canola oil treatments at P<0.05, P<0.01 and P<0.001, respectively.

Table 6—Chemical analyses of canola oil, with and without antioxidant, followed exposure to fluorescent light (7500 lux). Means[e] and standard errors

Characteristic	Storage time (hr)	Canola Oil Treatment: Untreated control (T1)	BHA/BHT (100 ppm ea) + MGC (T2)	AP (100 ppm) (T3)	AP (200 ppm) (T4)	AP (200 ppm) + MGC (T5)	SEM[f]
Peroxide value (meq/kg)	0	0.43	0.38	0.34	0.33	0.32	0.02
	8	4.44[a]	4.44[a]	3.66[b]	3.61[b]	3.44[b]	0.11***
	16	7.74[a]	7.56[a]	5.92[b]	5.57[b]	5.44[b]	0.15***
	24	13.79[a]	12.76[a]	9.85[b]	8.80[b]	8.82[b]	0.38***
TBA value[g] (452 nm)	0	4.93[b]	4.76[b]	6.03[a]	5.78[a]	5.77[a]	0.21**
	8	8.89	8.15	8.43	7.97	8.02	0.24
	16	10.49[a]	10.02[ab]	9.44[bc]	9.06[c]	8.70[d]	0.20**
	24	11.63[a]	11.34[a]	10.53[b]	10.24[b]	9.42[c]	0.16***
$E^{1\%}_{1cm}$ (234 nm)[h]	0	4.10	4.16	4.10	4.15	4.09	0.03
	8	4.28	4.26	4.20	4.20	4.20	0.03
	16	4.44	4.43	4.35	4.28	4.28	0.05
	24	4.62[a]	4.59[a]	4.36[b]	4.37[b]	4.28[b]	0.04**

[abcd] Means within the same row sharing a common letter are not significantly different at P≥0.05.
[e] Means are averages of 6 determinations (2 per each of 3 replicates).
[f] Standard error of the mean.
[g] Thiobarbituric acid value.
[h] Not corrected for triglycerids absorption.
*,**,*** Significant differences among canola oil treatments at P<0.05, P<0.01 and P<0.001, respectively.

increased protection from oxidation. Our data suggested that AP efficacy was not enhanced by the addition of MGC.

Fluorescent light test

The effects of AP and BHA/BHT + MGC on the sensory quality of canola oils exposed to fluorescent light (7500 lux for up to 24 hr) are shown in Table 5. After storage, odor and flavor scores for each of the illuminated samples were lower than those for T6. After 24 hr, samples treated with 200 ppm AP had a significantly less intense overall odor than the other oil treatments. However, the addition of AP (at 100 or 200 ppm) to canola oils did not retard development of off-flavor in the fluorescent light exposed samples. In contrast, McConnell and Esselen (1947) noted that AP inhibited off-flavor development in illuminated corn and cottonseed oils.

The predominant odor note detected in oils subjected to fluorescent light storage was rancid. The addition of AP to canola oil inhibited rancid odor development up to 8 hr. Slightly intense rancid odors (2 on the 6-point scale) were present in all samples following 16 hr illumination. The predominant flavor notes detected by panelists were fishy, grassy, painty, and rancid. Antioxidant (either BHA/BHT + MGC or AP with or without MGC) addition to canola oil did not inhibit production of the off-flavor notes.

Chemical determinations of oil quality (PV, TBA, and absorbance ($E^{1\%}_{1cm}$) at 234 nm) (Table 6) generally showed that AP inhibited canola oil photooxidation to some extent. However, numerical differences among treatments were minimal when compared to those obtained for Schaal oven data. After 24 hr exposure, oils treated with AP (T3, T4, and T5) had lower PV, TBA, and absorbance (234 nm) values than the untreated control (T1) and the BHA/BHT + MGC treated (T2) oils. In an earlier investigation of canola oil quality, Hawrysh et al. (1988) also found few treatment differences for antioxidant treated canola oils subjected to fluorescent light tests. Correlation coefficients for both accelerated storage tests (Table 7) showed highly significant relationships between sensory and chemical data. Negative correlations were as expected. All coefficients were excellent (above 0.76) or good (between 0.51 and 0.75) (Leporiere, 1976).

Table 7—Pearson correlation coefficients (r) between sensory evaluation data and chemical data for accelerated storage tests

Test	Schaal oven storage (N = 60) Odor scores	Schaal oven storage (N = 60) Flavor scores	Fluorescent light storage (N = 60) Odor scores	Fluorescent light storage (N = 60) Flavor scores
Peroxide values	−0.94***	−0.88***	−0.87***	−0.86***
TBA value[a] (452 nm)	−0.78***	−0.76***	−0.91***	−0.91***
$E^{1\%}_{1cm}$ (234 nm)	−0.94***	−0.88***	−0.74***	−0.73***
Total volatiles	−0.93***	−0.87***	—	—
Pentane/Butane	−0.94***	−0.90***	—	—
2,4-Decadienals	−0.87***	−0.81***	—	—

[a] Thiobarbituric acid value.
*** Significant at P<0.001.

CONCLUSIONS

THE SCHAAL OVEN storage test indicated that 200 ppm AP, with or without MGC, was effective in promoting canola oil stability in oils subjected to accelerated storage at 65°C for up to 12 days (Schaal oven test). Addition of BHA/BHT + MGC to canola oil resulted in a slight improvement in canola oil stability during storage. Results from the fluorescent light test

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Extraction of Annatto Seed Pigment by Supercritical Carbon Dioxide

A.J. DEGNAN, J.H. VON ELBE, and R.W. HARTEL

ABSTRACT

Operating pressures ranged 3000–7000 psi and temperatures 40–55°C. Maximum solubility of pure bixin was 0.003 and of annatto seed pigment 0.26 mg/g CO_2. Analyses of annatto seed pigment from supercritical carbon dioxide extractions showed only α- and β-bixin present. Only bixin was extracted from mixed samples consisting of bixin and norbixin. No thermal degradation product was detected in samples extracted within the temperature range we used. Increased temperature resulted in increased pigment/g CO_2 at all pressures. Isothermal increases in pressure did not increase pigment/g CO_2. Efficiency of annatto pigment extraction was increased in the presence of an oil.

INTRODUCTION

THE NUMBER of certified food colorants, as defined under the 1960 Color Additive Amendment to the Food, Drug and Cosmetic Act of 1938, is limited. In recent years synthetic dyes have been closely scrutinized for their potential toxic hazard (Ingram and Francis, 1969; Bhalkar and Dubash, 1983). The lake of FD&C Red Number 3, for example, is the latest food color removed from use because of safety. Hence, natural colorants, exempt from certification, are of interest.

Annatto is a carotenoid-type food colorant which occurs naturally in the seed of the tropical annatto tree (*Bixa orellana*). Organic solvents such as chloroform, dichloroethane, or acetone are used commercially to extract pigment from the seed. Heat is generally applied to the extract to reduce solvent residue within tolerable limits. High temperatures (>50°C) are known to degrade the pigment to yellow artifacts of low tinctorial strength (Mckeown and Mark, 1962).

The use of supercritical carbon dioxide (SC-CO_2) as solvent in annatto seed pigment extraction has the potential for precluding need for organic solvents or elevated processing temperatures. The objectives of our study, were to determine the solubility of annatto pigments in SC-CO_2 and to determine the quality of annatto pigment obtained with a SC-CO_2 extraction process.

MATERIALS & METHODS

Materials

Carbon dioxide, 99.9% pure, was obtained in 50-lb cylinders from Liquid Carbonic (Chicago, IL). Analytical Reagent grade chloroform was obtained from OmniSolv (Gibbstown, NJ). Annatto seeds and 97% pure bixin was donated by Chr. Hansen's Laboratory, Inc. (Milwaukee, WI).

Extraction

Figure 1 shows a schematic of the SC-CO_2 extraction apparatus. All parts contacting solvent and/or solute were machined from 316 stainless steel. Annatto seed (3g), or bixin (97% pure, 0.5g) were placed into the 11.8 ml vessel. Whole seeds were used because previous experiments using crushed seeds or glass beads (for increased sample surface area) did not increase concentration of pigments in SC-CO_2. Likewise, the pigment concentration from a sample of pure bixin was not increased by packing in glass beads. Soybean oil was used as an entrainer and, when included in the system, about 50 mL were placed into the 300 mL vessel. The system was then pressurized with CO_2 (between the compressor and valve V_1). System temperature equilibrated within 30 min.

Sample collection was accomplished by gently opening the inlet valve (V_1) and allowing solvent to flow into the sample chamber at a rate which enabled the pump to maintain extraction pressure. V_1 remained open until the sample chamber reached extraction pressure and was filled with solute-saturated SC-CO_2. Closing V_1 partitioned the sample between V_1 and V_2. The extract was brought back to atmospheric pressure by slowly opening valve V_2, allowing escape of gaseous CO_2 and precipitation of the solute in the chamber. Traces of pigment carried by the released CO_2 were recovered when the outlet gas passed through a 250 mL Erlenmeyer flask containing $CHCl_3$. Additional $CHCl_3$ (about 45 mL) was used to wash solute from the sample chamber. The pigment contained in the $CHCl_3$ represented the amount extracted by 6.80 mL of SC-CO_2 at operating P at the given conditions. The $CHCl_3$ was used only for thorough recovery of the extracted pigment. It did not contribute to extraction and would not be required in a continuous system.

Time allowed for equilibration between solvent and solute was determined empirically by analyzing the extract periodically until a maximum concentration was reached and maintained.

Analytical methods

The method of Mckeown and Mark (1962) was used in determining seed pigment content, calculated as % (wt) bixin. The method involved repeated extractions of seeds with $CHCl_3$. The total extract was measured and the pigment content was calculated based on light

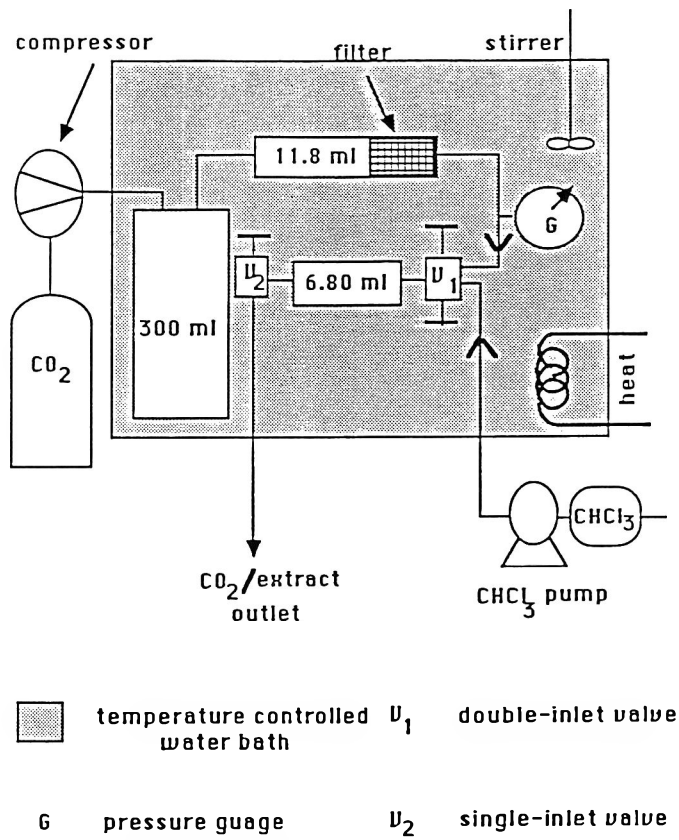

Fig. 1—Supercritical fluid extraction apparatus.

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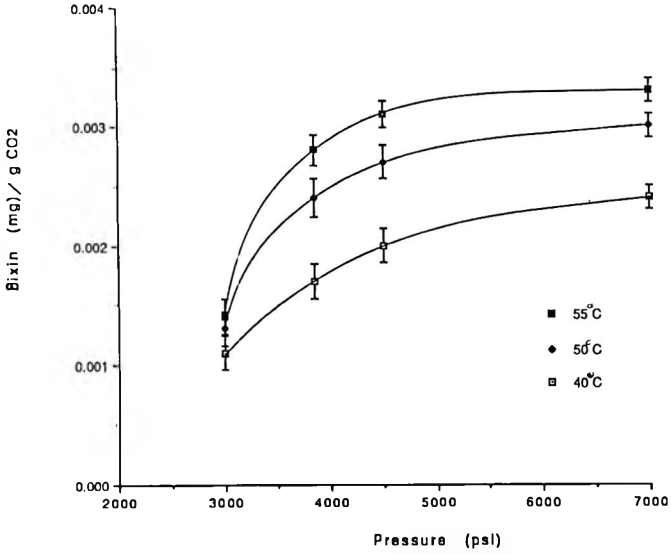

Fig. 2—Isothermal solubilities of pure bixin (97%) in CO_2.

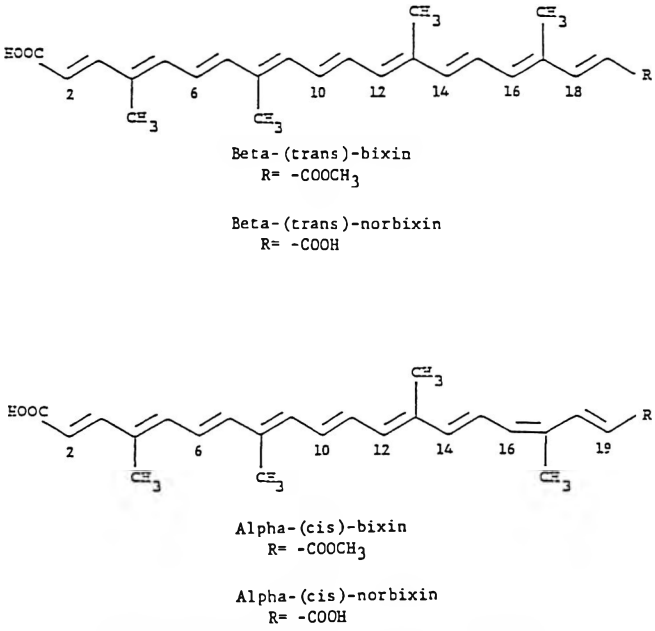

Fig. 3—Molecular structures of α-, β-bixin, and norbixin.

absorption of the extract at 501 nm and application of a 1% absorptivity value of 2826.

Seed oil content was quantified by extracting about 2g of finely ground seeds with 50 ml $CHCl_3$ while stirring and heating at 40°C. Five such extractions were sufficient to remove all $CHCl_3$ extractable material. The combined extracts were filtered through Whatman no. 1 filter paper and the total volume was measured. Ten mL portions of the total volume were evaporated to dryness in aluminum weighing pans for gravimetric analysis. The seed oil content was expressed as a percentage of weight of chloroform extractable material.

The concentration of bixin extracted by SC-CO_2 was determined by first dissolving the extracted pigment in $CHCl_3$ and calculating the content as described. The following equation was then used to calculate the concentration as mg bixin/g SC-CO_2.

$$\text{bixin (mg/g } CO_2) = (a \times 1 \times 10^3)/(A \times 6.8 \times d) \qquad (1)$$

where a = absorbance at 501 nm; 1 = total volume (liters) of the $CHCl_3$ used to wash the sample collection vessel; 10^3 = conversion from (g) bixin to (mg) bixin; A = 1% absorptivity value 2826 (liters $CHCl_3$/10g bixin); 6.8 = volume (mL) of sample collection vessel; d = density (g/ml) of CO_2 at extraction P and T for conversion to g CO_2 (Weast, 1970).

The quantity of annatto seed oil extracted with SC-CO_2 was measured as described by determining gravimetrically the amount of oil in 10 mL of the $CHCl_3$ used to dissolve the extracted pigment. The pigment to oil was about 1/100 by weight and therefore pigment weight was ignored in the calculation.

$$\text{Seed oil (g/g}CO_2) = (v \times s)/(.01 \times 6.8 \times d) \qquad (2)$$

where v = total volume (liters) of $CHCl_3$ to dissolve the extracted sample; s = weight (g) of residual oil after evaporation of $CHCl_3$; .01 = sample volume (liters); 6.8 = volume (mL) of sample vessel; d = density (g/ml) CO_2 at extraction P and T for conversion to g CO_2.

Norbixin was prepared by combining 1g pure bixin with 200 mL 5% potassium hydroxide (KOH) in ethanol (EtOH). The mixture was refluxed 15 min, cooled, and acidified with acetic acid. Resulting red crystals were further purified by washing with diethyl ether, followed by washing with 1% acetic acid. The crystals were then dissolved in diethylformamide (DEF) and methanol (MeOH) (1:1) for HPLC analysis.

Methyl bixin was synthesized by adding 1g bixin to 3g diazomethane in chloroform ($CHCl_3$). The solution was evaporated at ambient temperature and the red/purple crystals were dissolved in DMF and MeOH for HPLC analysis.

The reverse phase HPLC method of Smith et al. (1983) was used to separate α- and β-bixin, norbixin, and methyl bixin. A mobile phase of MeOH/2% acetic acid (8.7:1.3) at 1 mL/min was used with a stationary phase Nova Pak C_{18} Radial Pak Cartridge column (Waters Assoc. Co., Milford, MA). Twenty μL of a dilute solution of equal amounts of norbixin, bixin, and methyl bixin in DEF/MeOH (1:1) were injected into the HPLC mobile phase to determine retention times. A Vari-Chrom VUV-10 (Varian Assoc., Palo Alto, CA) variable wavelength detector at 500 nm monitored the pigment. Chromatograms were recorded on a series A-5000 Omniscribe strip chart recorder (Houston Instrument, Austin, TX).

The C_{17} degradation product had a maximum light absorbance at 403 and 428 nm (Mckeown, 1961) and therefore was not detected in the HPLC analysis. Detection was achieved by obtaining a visible light absorption spectrum between 520 and 370 nm using a Bausch and Lomb 2000 (Rochester, NY) spectrophotometer.

RESULTS & DISCUSSION

Pigment or oil composition of annatto seeds

Annatto seeds used in this study had been stored for 10 months at 2°C. The pigment and oil contents were 1.37% ± 0.09 (wt) and 11.6% ± 0.3 (wt), respectively. This pigment value was within the 0.76–3.70% range reported by Dendy (1966) and Bhalkar and Dubash (1983).

Solubility of bixin (97% pure) in SC-CO_2

Figure 2 represents the isothermal solubility of bixin (97% pure) in SC-CO_2 at 40, 50, and 55°C. Bixin concentrations ranged from 0.0010 ± 0.0001 mg bixin/g CO_2 at 3000 psi, 40°C to 0.0033 ± 0.0002 mg bixin/g CO_2 at 7000 psi, 55°C. These values were low compared to solubilities of some food components in SC-CO_2, such as hop resin (3.4 mg/g CO_2)(Vitzthum et al., 1978), water (1 mg/g CO_2), lactic acid (5 mg/g CO_2), and glycerol (0.5 mg/g CO_2) (Blenford, 1983). The three isotherms were nonlinear and pigment solubility at any pressure increased with temperature. Extent of temperature effect on solubility however varied at any pressure studied. The difference in solubility at 40 and 55°C at 3000 psi was only 25% while at 4000 it reached 60% and at 7000, 30%. McHugh and Paulaitis (1980) reported similar solubility characteristics for biphenyl in SC-CO_2.

Analysis of bixin solubilized by SC-CO_2

Bixin, $C_{25}H_{30}O_4$, is a twenty-carbon conjugated chain with a terminal carboxyllic acid group and a terminal methylester group (Fig. 3). Isomerization around the 15,16-carbon atoms results in trans and cis forms, called β and α, respectively (Zechmeister and Escue, 1944). Other derivatives of bixin include norbixin, which has a carboxyllic acid group at each

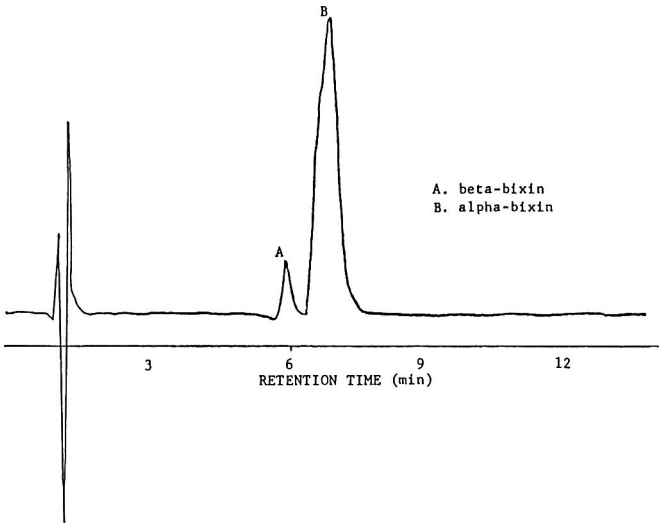

Fig. 4—HPLC chromatogram of pigment extract from sample of bixin and norbixin by SC-CO_2, 4300 psi, 50°C.

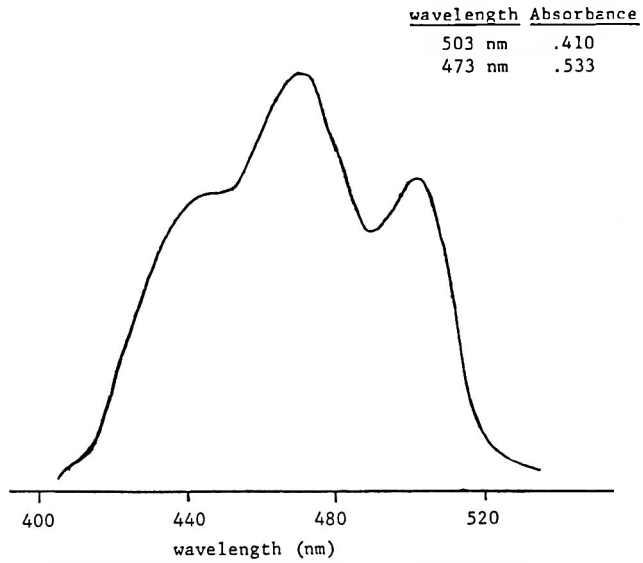

Fig. 5—Light spectrum of pigment from pure bixin (97%) solubilized by SC-CO_2, 4300 psi, 50°C.

terminal end of the twenty-carbon conjugated chain; methyl bixin, which has a methylester group at each terminal end; and the thermal degradation product of bixin, 4,8-dimethyltetradecahexaenedioic acid monomethylester.

An HPLC chromatogram of the extracted bixin sample is shown in Fig. 4. Peaks shown are characteristic for α- and β-bixin, based on our retention times of 2 min 50 sec for α-, 4 min for β-norbixin; 6 min 10 sec for α-bixin; and 7 min 5 sec for β-bixin. A spectrophotometric scan, between 520 and 400 nm, of the extracted pigment is shown in Fig. 5. The spectrum was similar to that reported by Mckeown and Mark (1962) for pure bixin. The absence of visible light absorption in the 400 nm region by the SC-CO_2 solubilized pigment indicated no thermal degradation product of bixin was present.

The solubility of bixin derivatives in SC-CO_2 was of interest to ascertain the influence of solute polarity. A sample was prepared containing 0.5g of the more polar norbixin (compared to bixin) and 0.5g of the less polar methyl bixin and subjected to SC-CO_2 extraction. Methyl bixin is the dimethyl ester of bixin and does not occur naturally in annatto seeds. The HPLC chromatogram of the sample after extraction is shown in Fig. 6. The chromatogram suggested that norbixin was not extracted under the experimental conditions and that methyl bixin,

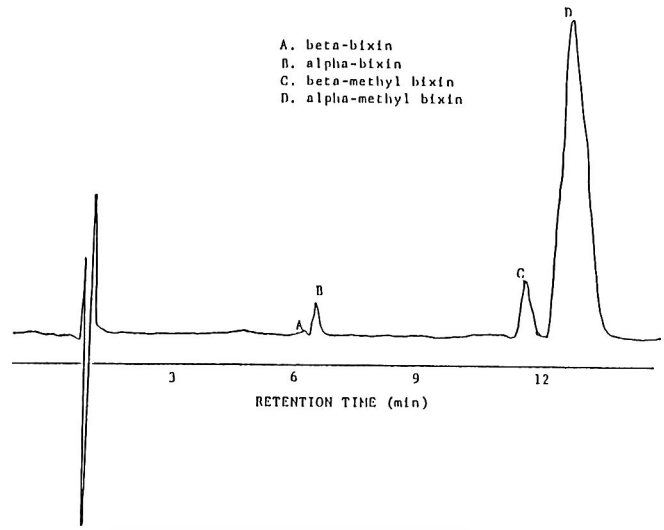

Fig. 6—HPLC chromatogram of pigment from sample of bixin and methyl bixin by SC-CO_2, 4300 psi, 50°C.

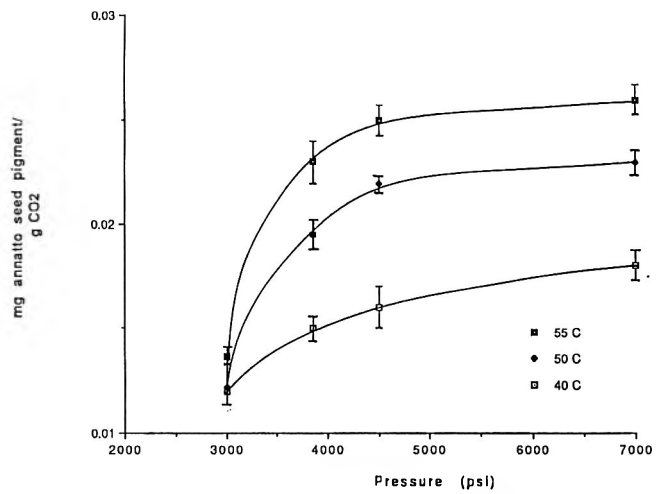

Fig. 7—Isothermal solubilities of annatto seed pigment in CO_2.

based on peak heights, was extracted in greater quantities than bixin. The concentration of methyl bixin in SC-CO_2 at 4300 psi and 50°C was calculated as 0.25 ± 0.02 mg methyl bixin/g CO_2. This was about 100 times gerater than the concentration of pure bixin extracted under the same conditions. The result shows that SC-CO_2 solubilized nonpolar substances preferentially over polar substances, and suggested that if annatto seeds were extracted, only bixin would be solubilized. The compound polarity data agreed with those reported by Rizvi (1986).

Solubility of seed pigment in SC-CO_2

Figure 7 shows the isothermal solubility of annatto seed pigment after the first pass of SC-CO_2 at 40, 50, and 55°C. The shape of the isotherms and the effects of temperature on pigment solubility were the same as those for pure bixin. The concentration of pigment in SC-CO_2 however, was about 10 times greater when annatto seeds, compared to pure bixin (97%), were used. Concentrations of annatto seed pigment ranged between 0.013 ± 0.001 at 3000 psi, 40°C to 0.027 ± 0.002 mg bixin/g CO_2 at 7000 psi, 55°C.

The increase in solubility of the pigment when annatto seeds were used was attributed to the seed oil extracted simultaneously. Bixin is relatively soluble in edible oils and commercial preparations contain up to 1.5% bixin (Bauernfeind and Klaui, 1981). Concentrations of annatto seed oil in SC-

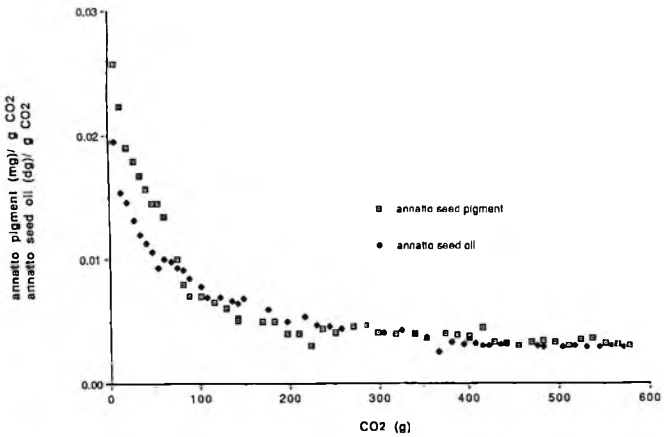

Fig. 8—Annatto seed oil or pigment in CO_2, 4300 psi, 50°C with consecutive sampling. Solvent flow rate 0.3g CO_2/min.

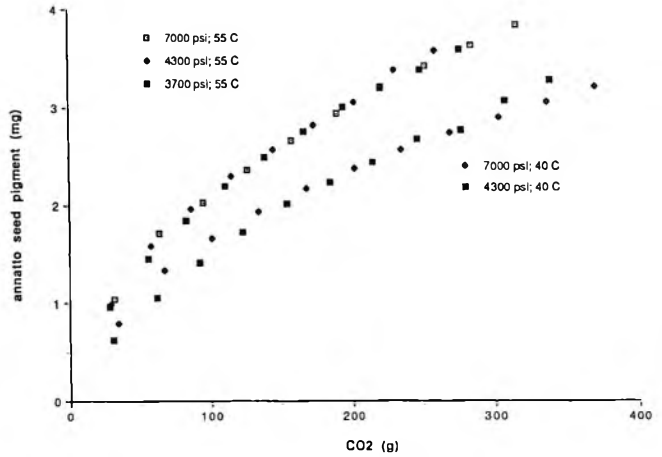

Fig. 9—Recovered annatto seed pigment as function of CO_2 used during SC-CO_2 extractions at isobaric and isothermal conditions.

Table 1—Annatto seed pigment or oil in supercritical carbon dioxide at various conditions of pressure and temperatures

	Annatto seed					
	Pigment (mg/g CO_2)[a]			Oil (mg/g CO_2)[a]		
psi	40°C	50°C	55°C	40°C	50°C	55°C
3000	0.011	0.012	0.014	0.3	0.5	0.6
3850	0.016	0.020	0.023	1.3	1.7	2.0
4500	0.017	0.023	0.026	1.5	1.9	2.3
7000	0.018	0.024	0.027	1.7	2.1	2.5

[a] ± 0.001

CO_2 ranged from 0.030 ± 0.001 at 3000 psi, 40°C to 0.026 ± 0.002 dg oil/g CO_2 at 7000 psi, 55°C. Table 1 shows pigment and oil concentrations of samples extracted at each condition of pressure and temperature.

Figure 8 shows the concentration of pigment and oil with consecutive sampling when annatto seeds were extracted at 4300 psi, 50°C. The parallel decrease in concentration of both oil and pigment as extraction proceeded was evidence that pigment solubility in CO_2 was influenced by presence of seed oil. The oil concentration was 100 times that of the pigment concentration. As oil concentration approached zero, the concentration of pigment or oil reached a steady state. The concentration of pigment approached a value similar to that of pure bixin in SC-CO_2. About 50% of total seed oil was extracted after 130g of CO_2 had been used. An additional 200g was required to remove 75%. We presumed that the concentration of oil decreased with each sample taken, because the readily available oil near the surface was depleted and the distance of oil migration through the seed increased.

Isothermal and isobaric extraction of seed pigment

Figure 9 shows the total pigment recovered from annatto seeds with sequential SC-CO_2 extractions under isobaric and isothermal conditions. Data show changes in extraction pressure did not change quantity of pigment/g CO_2 at isothermal conditions. However at each pressure studied the pigment concentration (or extraction efficiency) was affected by temperature, (275g SC-CO_2 at 55°C, compared to 40°C, extracted about 25% more pigment).

Data implied that removal of pigment or oil was independent of pressure during supercritical fluid extractions. However, extraction pressure influenced the rate of solute removal. Concentration of solute per unit volume CO_2 increased at conditions of higher CO_2 density (ie. 7000 vs 3700 psi). Therefore, at constant mass/volume flow rates the extraction pressure which produced the greatest mass of CO_2/unit voume would contain the most pigment/sample.

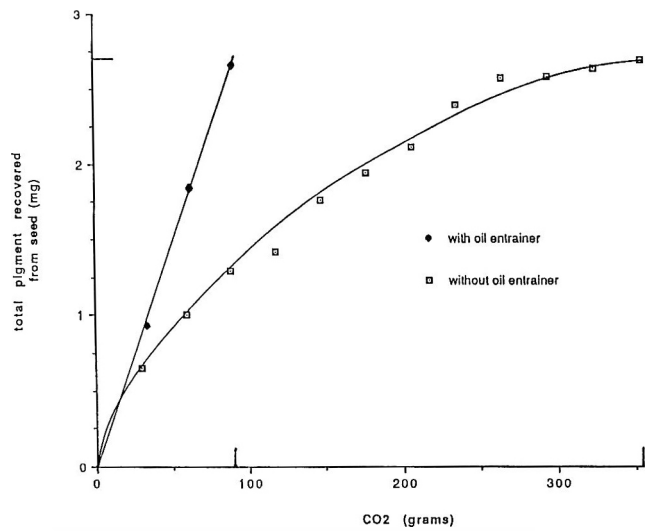

Fig. 10—Extracted annatto seed pigment 4300 psi, 50°C with and without soybean oil entrainer.

Analysis of seed pigment by SC-CO_2 extraction

The HPLC chromatogram and the spectrophotometric scan of annatto seed pigment obtained by SC-CO_2 extraction were similar to those shown in Fig. 4 and 5. Peaks in the chromatograms, based on retention times, were characteristic of α- and β-bixin and the lack of other peaks indicated other forms of bixin were not extracted. The spectra were similar to those for α- and β-bixin and the absence of light absorption at 400 nm indicated that the C_{17} thermal degradation product was not present.

Solubility of seed pigment in SC-CO_2 with oil entrainer

An oil entrainer was included in the extraction system because an increase in concentration of annatto seed pigment in SC-CO_2 was attributed to the presence of annatto seed oil. Soybean oil was selected as an oil entrainer from a group of edible oils, monoglycerides, and diglycerides currently used in annatto preparations (Preston and Rickard, 1980). Phase equilibration of soybean oil in SC-CO_2 occurred within 30 min and concentrations agreed with those determined by Stahl et al. (1980). Phase equilibration was accomplished in less than 1 hr when annatto pigment was extracted using the 11.8 mL vessel and SC-CO_2 at 2500 psi and 40°C. This method was tested using biphenyl and SC-CO_2 at 2200 psi and 45.4°C. Phase equilibration was accomplished in less than 1 hour and

concentrations of biphenyl in SC-CO_2 determined by this method agreed with those obtained by McHugh and Paulaitis (1980). The concentration of annatto seed pigment in SC-CO_2 at 4300 psi and 50°C with a soybean oil entrainer was 0.030 ± 0.002 mg pigment/g CO_2. Figure 10 shows the total pigment recovered at these conditions when seeds were extracted with and without an oil entrainer. Ninety grams of CO_2 were required to extract 2.7 mg pigment when the oil entrainer was used compared to 350g CO_2 with no entrainer. The consistency of pigment concentration during the extraction was attributed to the unlimited supply of entrainment oil compared to the decreasing availability of oil when the process depended on the seed oil. This consistency was also indicated by the linearity of the extraction curve. The concentration of oil extracted simultaneously with pigment by SC-CO_2 (4300 psi, 50°C) was calculated as 0.021 ± 0.002 dg oil/g CO_2.

The data suggested that use of an oil entrainer with SC-CO_2 extraction would increase extraction efficiency. Analysis of annatto seed pigment extracted by SC-CO_2 containing an oil entrainer, as with the previous extract, contained only α- and β-bixin and showed no traces of C_{17} degradation product.

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showed that AP had a limited effect in protecting canola oil from photooxidation, while BHA/BHT+MGC was ineffective.

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Optimization of Extraction of Peanut Proteins with Water by Response Surface Methodology

ISMAIL Y.S. RUSTOM, M.H. LÓPEZ-LEIVA, and BABOO M. NAIR

ABSTRACT

Water extraction of peanut proteins was optimized to maximize protein extraction, Y_1 and protein concentration in the extract, Y_2. A central composite design involving solids-to-water ratio (X_1), pH (X_2), temperature (X_3) and time (X_4) was used, and second-order models for Y_1 and Y_2 were employed to generate response surfaces. The optimum conditions to obtain $Y_1 \geq 85\%$ and $Y_2 \geq 2.5\%$ were X_1 = 1:8; X_2 = 8.0; X_3 = 50 °C; X_4 = 30 min. Estimates of Y_1 and Y_2 at the optimum were 85.29% and 2.7%, respectively. Experimental verification gave values of Y_1 = 84.39% ± 0.78 and Y_2 = 2.80% ± 0.02. Y_1 and Y_2 were further maximized, as measured by absorbance of solution following centrifugation of the extract, when papain (0.05% g/g peanuts) was added and extraction was carried out at the optimum conditions.

INTRODUCTION

THE SHORT SUPPLY and high cost of animal milk in some parts of the world, or lactose intolerant consumers have created a need for developing milk substitutes and nutritious beverages based on oilseeds and nuts (Swaminathan and Parpia, 1967). Peanut beverages have been studied using different preparation methods (Chompreeda et al., 1989; Rubico et al. 1989; Schmidt et al., 1978). The economic feasibility of such products depends partially on maximization of protein extraction from peanuts with water.

Extraction of peanut proteins with water was reported to be influenced by pH, ionic strength, pre-soaking, temperature, time and solids-to-water ratio (Elahi and Ali, 1971; Rhee et al., 1972). In a preliminary study at our laboratory, seven potential factors were screened for effects on water extraction of peanut proteins. Temperature, pH, solids-to-water ratio, and papain treatment of peanuts were reported significant in increasing solids extraction (Rustom et al., 1991). Increasing the water increased recovery of peanut solids in solution, but reduced solids in the extract. Optimization could be used to maximize extraction and solids concentration in the extract.

When many factors and interactions affect desired responses, response surface methodology (RSM) is an effective tool for optimizing the process (Hunter, 1959). RSM uses an experimental design such as the central composite design (CCD) to fit a model by least squares technique. If the proposed model is adequate, as revealed by the diagnostic checking provided by an analysis of variance (ANOVA) and residual plots, contour plots can be usefully employed to study the response surface and locate the optimum.

The purpose of our current work was to optimize extraction of peanut proteins with water, and to study the effect of optimum extraction conditions on the extent of hydrolysis of peanut proteins with papain.

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MATERIALS & METHODS

Materials

Peanuts, *Arachis hypogaea L.*, of unknown variety, imported from China (Asien Trading Co., Malmö, Sweden) were used as raw material for preparation of peanut extract. Papain (papainase; EC 3.4 22. 2), no. P 3375, (Sigma Chemical Co., Malmö, Sweden) was used to hydrolyze peanut proteins.

Experimental design

Three responses were measured: Percent of protein extracted (Y_1), defined as the ratio of total amount of protein in the extract (g) to total amount of protein in the peanuts (g) expressed as a percentage, concentration of protein in peanut extract (Y_2), and absorbance (400 nm) of solution phase following centrifugation of the extract (Y_3). Each of the variables to be optimized was coded at 5 levels: −2, −1, 0, 1, and 2. Table 1 shows the variables, their symbols and levels. The selection of variable levels was based on conclusions drawn from a previous study (Rustom et al., 1991).

A central composite design (CCD), shown on Table 2, was arranged to allow for fitting of a second-order model (Hunter, 1959). The CCD combined the vertices of a hypercube whose coordinates are given by the 2^n factorial design (runs 1–8 and 11–18) with the "star" points (runs 21–28). The star points were added to the factorial design to provide for estimation of curvature of the model (Joglekar and May, 1987). Six replicates (runs 9, 10, 19, 20, 29, and 30) at the center of the design were used to allow for estimation of the "pure error" sum of squares. Experiments were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors.

Statistical analysis

A software package (STATGRAPHICS, 1988) was used to fit the second-order models and generate response surface plots. The model proposed for each response (Y) was:

$$Y = b_o + \sum_{n=1}^{4} b_n X_n + \sum_{n=1}^{4} b_{nn} X_n^2 + \sum_{n \neq m=1}^{4} b_{nm} X_n X_m$$

Where b_0 is the value of the fitted response at the center point of the design, that is point (0,0,0,0). b_n, b_{nn} and b_{nm} are the linear, quadratic and cross-product regression terms respectively. The regression analysis was conducted using the "Stepwise Variable Selection—Backward Elimination" module (STATGRAPHICS, 1988). The criteria for eliminating a variable from the full regression was based on "F-to-remove" value of 2.0, 0.9 and 3.0 for Y_1, Y_2 and Y_3, respectively.

Table 1—Variables and their levels for central composite design

Variable	Symbol	Coded-variable levels[a]				
		−2	−1	0	1	2
Solids-to-water	x_1	1:6	1:7	1:8	1:9	1:10
pH	x_2	7.0	7.5	8.0	8.5	9.0
Temperature (°C)	x_3	30	35	40	45	50
Time (min)	x_4	10	20	30	40	50

[a] Passage from coded variable (X_i) level to natural variable (x_i) level is given by the following equations: $1{:}x_1 = 1{:}(X_1+8)$; $x_2 = 0.5X_2+8.0$; $x_3 = 5X_3+40$; $x_4 = 10X_4+30$.

Table 2—Central composite design arrangement and responses

Run	Variable levels[a]				Responses[b]		
	X_1	X_2	X_3	X_4	Y_1	Y_2	Y_3
1	−1	−1	−1	1	83.14	3.01	0.874
2	−1	−1	1	−1	82.67	2.90	0.975
3	−1	1	−1	−1	77.83	2.85	0.944
4	−1	1	1	1	86.79	2.13	1.000
5	1	−1	−1	−1	74.22	2.11	1.011
6	1	−1	1	1	85.41	2.46	1.533
7	1	1	−1	1	83.69	2.30	1.202
8	1	1	1	−1	83.91	2.33	1.962
9	0	0	0	0	83.17	2.61	1.013
10	0	0	0	0	79.39	2.48	1.020
11	−1	−1	−1	−1	80.78	2.85	1.209
12	−1	−1	1	1	82.41	3.07	1.572
13	−1	1	−1	1	85.51	3.09	1.089
14	−1	1	1	−1	80.65	2.93	1.052
15	1	−1	−1	1	84.47	2.34	0.868
16	1	−1	1	−1	84.75	2.25	0.873
17	1	1	−1	−1	81.41	2.18	1.261
18	1	1	1	1	85.55	2.33	1.187
19	0	0	0	0	83.57	2.57	0.764
20	0	0	0	0	84.00	2.58	1.070
21	2	0	0	0	86.33	2.18	0.966
22	−2	0	0	0	78.81	3.55	1.082
23	0	2	0	0	85.47	2.64	1.602
24	0	−2	0	0	83.04	2.61	0.768
25	0	0	2	0	83.62	2.75	0.985
26	0	0	−2	0	81.96	2.50	1.158
27	0	0	0	2	85.87	2.72	0.859
28	0	0	0	−2	81.72	2.38	1.187
29	0	0	0	0	84.55	2.68	0.791
30	0	0	0	0	85.65	2.63	1.181

[a] Coded variables.

[b] Y_1 = extracted protein (%) = (protein in the extract (g)/protein in peanuts used (g)) × 100. Y_2 = protein concentration in the extract (%). Y_3 = absorbance of solution following centrifugation of the extract.

Peanut extract preparation

Peanut kernels (5.00% moisture, 22.04% protein, 48.23% fat, 2.62% ash, and 22.11% carbohydrates) were manually blanched and ground twice in a kitchen meat mincer (Braun AG, Braun, Frankfurt, Germany). The water extracts were prepared by suspending 15g of meal in a beaker in distilled water according to the level of solids-to-water (see Table 1). Crude papain powder, activated by dissolving in water at 37°C, was then added to the suspension (0.05% g/g peanuts) to hydrolyze peanut proteins. The pH was adjusted with 1N NaOH or 0.1N HCl. Extraction temperature was controlled by positioning the beaker in a temperature-controlled water bath (Julabo Paratherm II, Juchheim Labortechnik, Schwarzwald, Germany). A mechanical stirrer continuously mixed the suspension. Conditions of each experiment are shown in Table 2. After treatment, the mixture was filtered through muslin cloth and then through an oil filter paper (Moulinex Microfilter System, Moulinex, France). The residual cake was discarded and the filtrate, (peanut extract) was stored at −10°C until analyzed.

Protein assay

Peanut extract was analyzed for nitrogen content using a Kjeltic Auto 1030 Analyzer (Tecator AB, Höganäs, Sweden). A Kjeldahl factor of 5.46 was used to calculate protein content (Woodroof, 1969). Results were expressed as % protein extracted (Y_1) and concentration of protein in the extract (Y_2).

Absorbance measurement

Preliminary absorbance measurements of solutions following centrifugation of hydrolyzed and unhydrolyzed extracts were made to establish adequate centrifugation conditions and best UV wavelength. Peanut extract contained in 10 mL-tubes were centrifuged at 5000 × *g* for 20 min at 20°C. A three-phase separation was obtained: top layer—fat, bottom layer—sediment, and middle layer—solution. The solution was transferred into disposable cuvettes. The absorbance of the solution was measured at 400 nm, using an UV-Visible Spectrophotometer (DMS 80, Varian Techtron Pty. Ltd., Mulgrave, Australia) vs distilled water.

RESULTS & DISCUSSION

Diagnostic checking of the fitted models

ANOVA for the regression was performed to assess the "goodness of fit." Only terms found significant by the ANOVA were included in the model. The models for Y_1, Y_2, and Y_3 were:

$$Y_1 = 83.25 + 0.78\,X_1 + 0.51\,X_2 + 1.02\,X_3 + 1.63\,X_4 - 0.30\,X_1^2 + 0.66\,X_1X_3 - 0.90\,X_3X_4.$$

$$Y_2 = 2.60 - 0.34\,X_1 + 0.01\,X_2 + 0.05\,X_3 + 0.08\,X_4 + 0.06\,X_1^2 - 0.02\,X_4^2 - 0.01\,X_1X_2 + 0.01\,X_1X_3 - 0.01\,X_1X_4 - 0.01\,X_2X_4.$$

$$Y_3 = 1.10 + 0.04\,X_1 + 0.10X_2 - 0.03\,X_4 + 0.12\,X_1X_2 - 0.10X_2X_4.$$

The result of ANOVA is shown on Table 3. The models fitted for Y_1 and Y_2 were significant by the F-test at the 5% confidence level. The lack of fit test measures the failure of the model to represent data in the experimental domain at points which are not included in the regression. None of the models exhibited lack of fit. The coefficient of determination (R^2) is the proportion of variability in the data explained or accounted for by the model (Montgomery, 1984). The coefficient of variation (CV) is the ratio of the standard error of estimate to the mean value of observed response expressed as a percentage. It is a measure of reproducibility of the models. As a general rule, a model can be considered reasonably reproducible if its CV is not greater than 10% (Joglekar and May, 1987).

Table 3 shows that models for Y_1 and Y_2 may represent the data and can be used to generate response surface plots for optimization. Although only 58.1% of variability in Y_1 (R^2 = 0.581) was accounted for by the model, it could still be accepted to represent the data since it was significant by the F-test at the 5% level. Furthermore, investigation of assumptions underlying the validity of the F-test (i.e. the residual is a random independent variable normally distributed with constant variance) confirmed that conclusion (see e.g. Montgomery, 1984).

The model fitted for absorbance (Y_3) was significant at the 10% level and had a low R^2 value and a relatively high CV. This may be attributed to difficulties in obtaining a representative sample for absorbance measurement. The CV of Y_3 indicated that the reproducibility of absorbance measurements was less than 80%. The model exhibited no lack of fit. Normal

Table 3—ANOVA for the fitted models

Source	Y1			Y2			Y3		
	df	ss	F	df	ss	F	df	ss	F
Model	7	131.69	4.36*	10	3.21	127.81*	5	0.67	2.23**
Residual	22	94.98		19	0.05		24	1.45	
Lack of fit	17	71.22	0.88	14	0.02	0.18	19	1.22	1.38
Pure error	5	23.67		5	0.03		5	0.23	
Total	29	226.67		29	3.26		29	2.12	
R^2		0.581			0.985			0.315	
CV(%)		0.02			1.90			22.32	

* Significant at the 5% level.

** Significant at the 10% level.

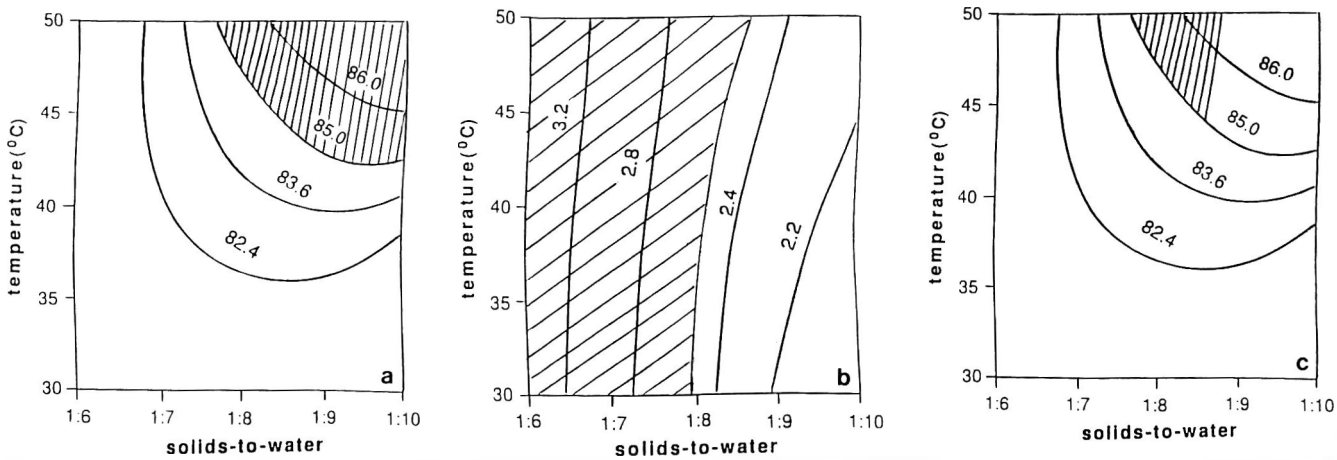

Fig. 1—(a) Response surface contours for percent of protein extracted (Y_1). Time = 30 min; pH = 8.0 Shaded area represents $Y_1 \geq 85\%$. (b): Response surface contours for concentration of protein in peanut extract (Y_2). Time = 30 min; pH = 8.0. Shaded area represents $Y_2 \geq 2.5\%$. (c): Overlap area (shaded) of superimposing contours of $Y_2 \geq 2.5\%$ over $Y_1 \geq 85\%$.

probability plot of residual, plot of residuals vs estimated values for Y_3, and plot of residuals vs random order of runs revealed that the residual satisfied the assumptions of normality, independence and randomness. Based on these we accepted model Y_3.

Response surface plotting

Variables giving quadratic and interaction terms with the largest absolute coefficients in the fitted models were chosen for the axes of the contour plots to account for curvature of the surfaces. Temperature and solids-to-water ratio were selected for the vertical and horizontal axes respectively for the contour plots of Y_1 and Y_2, while time and pH were measured at different levels. Contour plots considered in the optimization are shown on Fig. 1.

Optimization based on Y_1 and Y_2

The models Y_1 and Y_2 are useful in indicating the direction in which to change variables in order to maximize protein extraction and concentration of protein in the extract. The model for Y_1 indicated that temperature and time were the major variables affecting extraction of peanut proteins; they showed significant first order and two-factor interaction terms (X_3, X_4 and X_3X_4). The positive slopes: $b_3 = 1.02$ and $b_4 = 1.63$, indicated protein extraction increased with increased temperature and time. However, high temperature ($\geq$ 60 °C) is not recommended. Increasing temperature from 20 to 60 °C decreased protein extraction notably (Rustom et al., 1991).

Solids-to-water ratio (X_1) had significant effect on protein extraction (Y_1). Its positive slope ($b_1 = 0.78$) indicated protein extraction increased with increased solids-to-water ratio. Rhee et al. (1972) reported that peanut protein extraction could be increased if solids-to-water ratio was increased to 1:20. The model for Y_2 indicated that solids-to-water ratio was the most significant variable affecting concentration of protein in the extract; it had the greatest slope ($b_1 = -0.34$). The negative sign indicated that protein concentration in the extract decreased as solids-to-water ratio increased.

The pH (X_2) also had a significant effect on (Y_1) with a positive slope ($b_2 = 0.51$). Peanut proteins are 90% globulins and 10% albumins; their solubility in water can be increased by increasing pH above 7.0. However, above pH 8.2 no significant peanut protein solubilization could be achieved (Natarajan, 1980).

In a previous investigation we concluded that the optimization of the extract of peanut solids extraction with water for production of a peanut beverage could be based on the maximum amount of protein extracted and suitable beverage composition (Rustom et al. 1991). Figure 1(b), however, indicates that the solids-to-water ratio is crucial for protein content in the extract. An acceptable compromise utilized the following criteria: $Y_1 \geq 85\%$ [shaded area on Fig. 1(a)] and $Y_2 \geq 2.5\%$ [shaded area on Fig. 1(b)]. The region which satisfies both conditions was obtained by superimposing contour plot Y_2 over Y_1 to obtain the shaded area shown on Fig. 1(c). A combination of optimum working conditions can be selected from this area. The point at pH = 8.0, time = 30 min, temperature = 50°C, and solids-to-water = 1 : 8 can be recommended as a practical optimum. The estimated values for $Y_1 = 85.29\%$ and $Y_2 = 2.70\%$ were obtained at those conditions.

A verification experiment at the optimum conditions, consisting of 6 runs, was performed (following the described procedure of extract preparation) using 60 g peanuts in each run. The result was: $Y_1 = 84.39\% \pm 0.78$; $Y_2 = 2.80\% \pm 0.02$. The value obtained for Y_1 (84.39%) was less than the acceptance criterion ($Y_1 \geq 85\%$). By using "hypothesis testing" technique (Montgomery, 1984), this difference was shown to be nonsignificant at the 5% level. Comparing the value of Y_1 obtained in the verification experiment to the one estimated by the model for Y_1 indicated the model was 99% efficient.

Papain treatment

Enzymatic hydrolysis of peanut proteins prior to extraction was reported to increase their protein solubility in water due to degradation of large-molecular weight globulins, and to improve some functional properties most desired in beverage systems (Sekul and Ory, 1977; Sekul et al., 1978). Rustom et al. (1991) found that extraction of peanut proteins significantly increased when they were hydrolyzed with papain as compared to unhydrolyzed samples.

Change in absorbance is commonly used to detect extent of conversion in enzyme reactions (Dixon and Webb, 1958). Figure 2 indicated that the solution following centrifugation of unhydrolyzed extract had higher absorbance than solution from hydrolyzed extract at any wavelengths examined. This difference was maximum at 400 nm. Accordingly, increase in extent of hydrolysis of peanut proteins was detected by a decrease in absorbance at 400 nm.

In the model for Y_3, pH (X_2) was the major variable affecting absorbance; it had the greatest slope ($b_2 = 0.10$), and had significant interaction with solids-to-water ratio ($b_{12} = 0.12$) and time ($b_{24} = -0.10$). The pronounced effect of pH on extent of hydrolysis was attributable to its influence on papain activity. Enzyme activity changes with pH due to changes in ionization of the enzyme or the substrate. Crude papain has

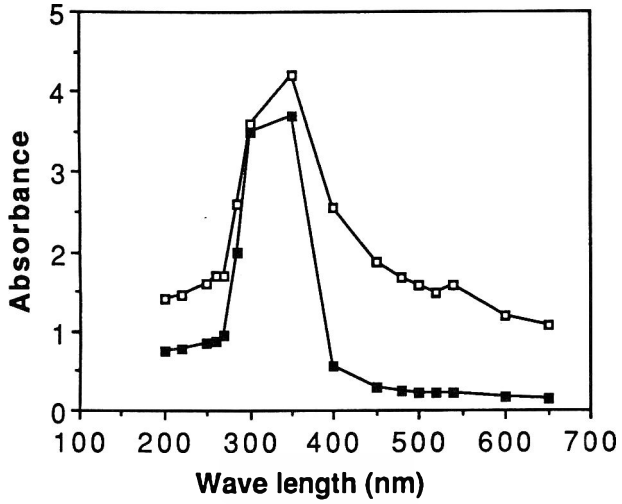

Fig. 2—Absorbance of solution following centrifugation (5000 × g; 20 min; 20°C) of peanut extract prepared using solid-to-water = 1:8; pH = 8.0; temp = 40 °C; time = 30 min. □—□ unhydrolyzed; ■—■ hydrolyzed (papain conc = 0.5%, g papain/g peanuts).

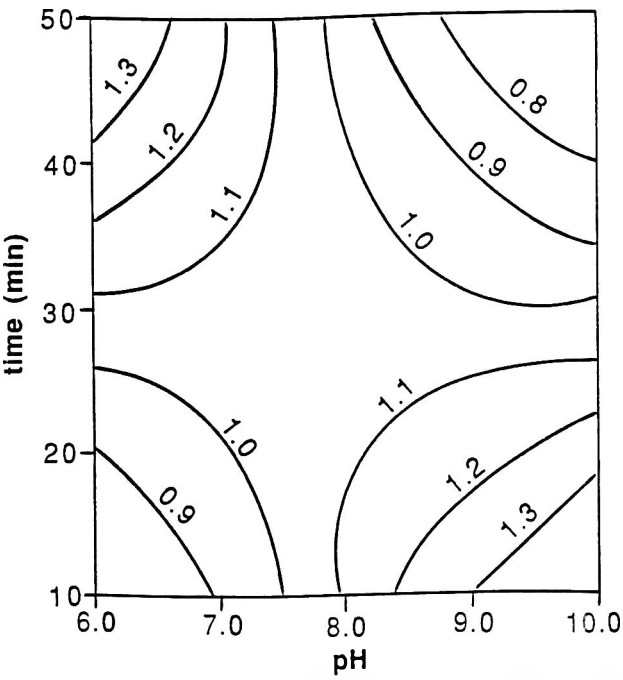

Fig. 3—Response surface contours for absorbance (400 nm) of solution following centrifugation of peanut extract (Y_3). Solids-to-water = 1:8.

a broad workable pH range (pH 5.0 to pH 9.0, Adler-Nissen, 1986).

Solids-to-water ratio (X_1) is a measure of substrate concentration, [S], for reaction of papain with peanut-water mixture; [S] is inversely proportional to X_1. The positive slope of X_1 ($b_1 = 0.04$) in the model for Y_3 revealed the dependence of extent of hydrolysis on substrate concentration; decreasing X_1 (i.e. increasing [S]) decreased the absorbance (i.e. increased extent of hydrolysis). This was in agreement with the Michaelis model describing the kinetics of enzyme reactions (Guilbault, 1970); for low-concentration substrates, the reaction rate was proportional to substrate concentration.

Time (X_4) had a negative slope ($b_4 = -0.03$) in the model for Y_3. This indicated that when time was increased absorbance was decreased (i.e. extent of hydrolysis was increased). However, increase in extent of hydrolysis is limited when equilibrium is reached. Sekul and Ory (1977) reported that hydrolysis of defatted peanut flour with papain (flour-to-water = 1:19; papain concentration = 0.5% w/v; temperature = 45 °C) reached equilibrium in 15 min.

The response surface of absorbance (Fig. 3) exhibited a "symmetrical saddle;" contours were symmetrical hyperbolas. There was a "minimax;" i.e. a minimum at the bottom of the saddle, whereas the surface tended to rise at the edges (Edgar and Himmelblau, 1988). The point at pH = 8.0; time = 30 min; solids-to-water = 1:8, which was recommended as the optimum, provided suitable conditions for hydrolysis. It was located in the bottom of the saddle where the absorbance was minimum; i.e. where extent of hydrolysis was maximum.

CONCLUSIONS

OPTIMUM EXTRACTION of peanut proteins with water for processing of peanut beverage can be achieved by extracting 1 part of peanuts with 8 parts water (w/v ratio) at pH = 8.0 and temperature = 50 °C for 30 min. Papain added to the mixture (0.05% g papain/g peanuts) increased solubilization of peanut proteins. Such conditions resulted in extraction of 84.39% of proteins contained in peanut solids, and protein concentration of 2.8% in the extract.

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Functional and Sensory Properties of Salad Dressing Containing Fermented Peanut Milk

CHAN LEE and LARRY R. BEUCHAT

ABSTRACT

The effects of substituting peanut milk fermented with mixed cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* for buttermilk on chemical, physical and sensory properties of ranch-style salad dressing were investigated. Increased amounts of fermented peanut milk in dressing resulted in decreased lightness, creamy flavor, oil emulsion capacity and consistency. Changes were dependent upon the commercial brand of dressing mix. Fermented peanut milk can be substituted for buttermilk in salad dressing at levels up to 25% without substantial reduction in sensory qualities.

INTRODUCTION

THE FUNCTIONAL PROPERTIES of legume proteins as affected by physical and chemical treatments have been studied extensively in attempts to develop new food ingredients (Beuchat et al., 1975; Puski, 1975; Quinn and Beuchat, 1975; Beuchat, 1977; Ahmed and Schmidt, 1979; Canella et al., 1984; Ponnampalam et al., 1987; Ahmed and Ramanatham, 1988). Changes in functional properties of peanut milk treated with enzymes have also been investigated (Chiou et al., 1985).

Peanut milk fermented with lactic acid bacteria has potential use as an ingredient in a variety of foods. It could be used alone or as a substitute for fermented dairy products such as buttermilk to enhance sensory qualities. One product in which fermented peanut milk might be successfully incorporated is salad dressing. Salad dressings can be defined as semi-solid foods generally containing edible vegetable oil, acidifying ingredients (usually vinegar or lemon juice), egg yolk, starch and specific seasoning or flavoring ingredients. A creamy mouthfeel and the unique fermented dairy flavors associated with salad dressings containing buttermilk are considered to be important sensory attributes. The distinct acid note characteristic of buttermilk is also desirable. Therefore, if fermented peanut milk is to be incorporated into salad dressing formulas, flavor as well as mouthfeel and other physical properties should not be adversely affected.

Chemical and sensory qualities of peanut milk change significantly upon fermentation with *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*. Major changes in headspace gas volatiles in fermented peanut milk include a decrease in hexanal content and an increase in acetaldehyde (Lee and Beuchat, 1991). These changes correlated with changes in sensory qualities as determined by trained panelists. Changes were accompanied by a significant decrease in green/beany flavor and a significant increase in creamy flavor. These observations enhanced the prospect of using fermented peanut milk as a substitute for buttermilk in salad dressings.

Emulsion capacity, foamability and water absorption of freeze-dried fermented peanut milk powder were studies by Schaffner and Beuchat (1986a,b). However, information on functional properties of fermented peanut milk when incorporated in food systems is meager. The objectives of our investigation were to determine the effects of incorporating fermented peanut milk into ranch style salad dressing on functional and sensory properties of the dressing and to compare these properties to those of dressings containing buttermilk.

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MATERIALS & METHODS

Seed and source

Florunner cultivar peanuts (*Arachis hypogaea* L.) were purchased from the University of Georgia College of Agriculture, Southwest Branch Experiment Station, Plains, GA. Upon receipt, seeds were stored at 7°C and 60% relative humidity until used.

Preparation of peanut milk

The procedure for preparing aqueous extracts of peanuts (peanut milk) is described in another report (Lee and Beuchat, 1991).

Bacterial strains and culture methods

Mixed cultures of *L. bulgaricus* (now *L. delbrueckii* subsp. *bulgaricus*) and *S. thermophilus* (now *S. salivarius* subsp. *thermophilus* were provided by Miles Inc. (Culture 9085; Biotechnology Products Division, Madison, WI) and Chr. Hansen's Laboratory (Culture 14128; Milwaukee, WI). Strains of each bacterium undoubtedly differed somewhat in metabolic capabilities; however, a comparison of biochemical characteristics was not made. Pure cultures were isolated, activated and adapted to a peanut milk based medium according to protocol described in a previous report (Lee and Beuchat, 1991).

Fermentation procedure

Active cultures served as inocula (1% each for each strain). Cultures were added to 200-mL quantities of sterile peanut milk which had been supplemented with 2% glucose and adjusted to 43°C. Fermentation was carried out at 43°C.

Formulation of salad dressing

Two commercial brands of powdered ranch-style salad dressing mix were purchased from a local supermarket. Brand A (Hidden Valley Ranch Dressing Mix, The HVR Co., Oakland, CA) contained salt, monosodium glutamate, maltodextrin, dehydrated garlic, dehydrated onion, dehydrated parsley, spice, carrageenan, calcium stearate, soybean oil and sulfiting agents; brand B (Good Seasoning Ranch Dressing Mix, General Foods Corp, White Plains, NY) contained corn syrup solids, salt, monosodium glutamate, cultured buttermilk solids, gum arabic, whey, modified corn starch, onion powder, garlic powder, lactic acid, parsley flakes, hydroxypropylmethylcellulose, spice, citric acid, whole milk solids, guar gum, partially hydrogenated cottonseed and soybean oil, and natural and artificial flavors. To 11g of brand A dressing mix and 25g of brand B dressing mix, 227g (8 oz) of buttermilk (brand A), whole fat cow's milk (brand B) or fermented peanut milk (brands A and B) and 227g of mayonnaise (Best Foods, CPC International, Inc., Englewood Cliffs, NJ) were combined and thoroughly mixed. Buttermilk and fermented peanut milk were adjusted to 8°C before formulations were prepared.

A third series of formulations was prepared using powdered brand C ranch dressing mix (Heller Seasonings and Ingredients, Inc., Bedford Park, IL), which contained salt, monosodium glutamate, mal-

Table 1—Intensity scores by a trained panel and preference ratings by an untrained panel for ranch-style salad dressings containing buttermilk, milk and fermented peanut milk

Type of panel	Sensory attribute	Brand A			Brand B		
		Butter-milk	Fermented peanut milk			Fermented peanut milk	
			9085[a]	14128[a]	Milk	9085[a]	14128[a]
Trained	Color						
	Whiteness	6.1	5.5	5.4	6.2	5.3	5.1
	Flavor						
	Creamy	4.2	4.9	4.7	4.8	3.8	3.9
	Beany/Green	0.9 c[b]	3.1 a	3.7 a	1.5 bc	2.3 ab	2.5 ab
	Spicy	6.6	6.2	6.4	6.0	5.6	5.7
	Sour	3.4	3.2	3.6	3.0	3.9	4.0
	Sweet	2.2	2.1	2.1	2.2	1.8	1.8
	Astringent	1.8	1.5	1.5	1.2	1.4	1.7
	Mouthfeel						
	Chalky	1.3	1.3	1.5	1.5	1.4	1.1
	Consistency	5.5 b	6.1 b	6.2 b	8.6 a	5.4 b	4.8 b
	Gummy	0.6	0.8	0.8	1.1	0.7	0.8
	Smoothness	2.8	4.3	4.2	3.9	4.4	3.1
Untrained	Appearance	10.5	10.7	10.6	10.0	10.6	10.2
	Color	11.0	11.5	11.6	10.8	11.0	10.7
	Flavor	9.6 a	7.9 b	7.5 b	8.7 ab	8.4 ab	8.2 ab
	Consistency	8.9 b	9.0 b	8.8 b	12.4 a	9.7 b	8.9 b

[a] Numbers (9085 and 14128) indicate sources of lactic acid bacteria cultures.
[b] Mean values in the same row which are not followed by the same letter are significantly different ($P \leq 0.05$).

todextrin, dehydrated onion, dehydrated parsley, spice, carrageenan, calcium stearate and soybean oil. The control formula consisted of 227g of buttermilk, 23 mL of vinegar (120 grain) and 11g (0.4 oz) of mix. Fermented peanut milk was substituted for buttermilk at 25, 50, 75, and 100% levels to result in four test formulations. All formulations were adjusted to 8°C before subjecting to chemical, physical and sensory analyses.

Analyses

pH and titratable acidity. The pH of dressings was determined using an Accumet pH meter (Model 805MP; Fisher Scientific Co., Pittsburgh, PA). The titratable acidity was measured by titrating buttermilk, fermented peanut milk and formulated salad dressings with 0.1 N NaOH using 1% phenolphthalein as an indicator. Titratable acidity was calculated and expressed as percent lactic acid.

Color. Color was measured using a Gardner Colorimeter (Pacific Scientific Co., model XL 800, Gardner Laboratory Division, Bethesda, MD) equipped with an XL 845 circumferential sensor. The reference plate used for salad dressing was of white hue with color coordinate values of $L = 94.11$, $a = -0.99$ and $b = 0.89$.

Emulsion capacity. Emulsion capacity was determined by a modified procedure adapted from those described by Carpenter and Saffle (1964) and Inklaar and Fortuin (1969). Buttermilk or fermented peanut milk (138 mL, combined volume, in ratios of 1:0, 3:1, 1:1 and 1:3 [percentages of 100/0, 75/25, 50/50 and 25/75, respectively]), 7g of brand C ranch dressing mix and 14 mL of vinegar (159g of base formulation) were deposited in a glass jar (500 mL) in which a 1-cm diam hole had been cut in the bottom. Peanut oil (Planters LifeSavers Co., Winston-Salem, NC) was added at the rate of 0.3 mL/sec from a 100-mL buret through the 1-cm diam hole in the inverted blender jar and blended at low on an Osterizer blender (No. 965-04F). The emulsion capacity was defined as the minimum amount of oil needed to reach the emulsion breakpoint. The emulsion breakpoint was subjectively defined as a sudden loss in the apparent consistency of the emulsion.

Sensory evaluation of salad dressing. Descriptive sensory analysis of ranch style salad dressings prepared from brand A, brand B and brand C mixes was conducted using ten trained panelists. Sensory attributes were rated for intensity using a 15-cm unstructured scale. Standards representing five flavor intensities were provided to each trained panelist for judgment of flavor terms (Meilgaard et al., 1987) characteristic of raw fermented milk, i.e., creamy, beany/green, spicy, sour, sweet, and astringent; the anchor for each characteristic was weak/strong (0–15). Ratings for color using a white tile as a standard and mouthfeel using milk of magnesia as a standard were also determined using the 15-cm scale. Anchors for mouthfeel characteristics were chalky (absent/present), consistency (thin/thick), gummy (absent/strong) and smoothness (smooth/lumpy). Anchor points for color (whiteness) were light/dark. Preference ratings for brands A and B dressing formulas were done using twenty-four ungrained panelists. A 15-cm unstructured scale (0 = dislike, 15 = like) was used.

Consistency. Consistency (apparent viscosity) of ranch style salad dressing was determined using a RVT Synchro-lectric Brookfield viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA) equipped with a T-spindle number TB and a Helipath stand. The spindle speed was 50 rpm and the maximum depth of penetration was 3 cm. The viscometer dial reading was converted to centipoise units by multiplying it by the manufacturer-supplied factor of 80. The data obtained with the T-spindle were not suited for rigorous mathematical analysis involving shear rates and stresses; however, data can be used for relative comparison of viscosity characteristics of a liquid as long as the same spindle and rpm settings are employed (Cooley et. al., 1954).

Emulsion stability. The stability of emulsions prepared from salad dressing formulas was determined. Dressings were deposited in 100-mL graduate cylinders and the volume (mL) of fluid which separated on the top of the emulsion phase was recorded over a 21-day period at 5°C.

Statistical analysis. Values reported represent means of three replicate trials. A Statistical Analysis System (SAS, 1985) program was used to analyze data. Analysis of variance (ANOVA) was done initially to determine the main and interaction effects. For experiments involving dressings prepared from brand A and brand B mixes, significance of mean differences was determined by Duncan's Multiple Range test. For experiments designed to evaluate the effects of partial substitution of fermented peanut milk for buttermilk (brand C formulations), when F values were significant, mean differences were compared by using least significant differences (LSD) at the 5% level of probability.

RESULTS & DISCUSSION

THE WHITENESS of ranch-style salad dressings was unaffected by the presence of fermented peanut milk in dressings prepared from brand A and brand B dry mixes (Table 1). The beany/green flavor in brand A salad dressing containing fermented peanut milk produced using both types of lactic cultures (9085 or 14128) was scored significantly higher compared to the brand A buttermilk and brand B milk control formulations. No significant differences in beany/green flavor were noted in brand B dressing when fermented peanut milk was substituted for milk. The consistency (a texture attribute) of the control formulation of brand B salad dressing, which contained cow's milk, was significantly thicker than that of all other formulations as determined by both trained and untrained panelists. This was in agreement with results from instrumental analysis (Table 2).

With the exception of consistency and beany/green flavor,

Table 2—Physical and chemical measurements for ranch style salad dressing containing buttermilk, milk and fermented peanut milk

	Brand A			Brand B		
		Fermented peanut milk			Fermented peanut milk	
	Buttermilk	9085[a]	14128[a]	Milk	9085	14128
Consistency (cps)	17.80 d[b]	14.20 d	16.90 d	93.20 a	28.70 c	35.40 b
Color						
L	87.92 ab	88.37 a	88.06 a	85.75 d	87.02 c	87.20 bc
a	−4.13	−4.09	−4.02	−3.36	−3.62	−3.43
b	14.35 b	13.57 c	13.56 c	15.21 a	13.94 bc	13.95 bc
Chroma	14.95 ab	14.18 b	14.15 b	15.58 a	14.40 b	14.37 b
Hue	105.92	106.72	106.45	102.43	104.55	103.80
pH	4.50 ab	4.48 ab	4.53 a	4.42 b	4.03 c	4.01 c
Titratable acidity (%)	0.60 c	0.36 d	0.34 d	0.70 b	0.76 a	0.76 a

[a] Numbers (9085 and 14128) indicate sources of lactic acid bacterial cultures.
[b] Mean values in the same row which are not followed by the same letter are significantly different ($P \leq 0.05$).

trained panelists did not detect significant differences in sensory attributes of the six formulations (Table 1). Untrained panelists rated flavor of brand A dressings containing fermented peanut milk significantly lower in intensity compared to the buttermilk control. Untrained panelists were unable to detect significant differences in appearance and color of the six formulations (Table 1).

The significantly higher consistency values for brand B formulas were apparently due to the thickening effect of hydroxypropylmethylcellulose and guar gum (Table 2). Lightness (L) of salad dressing containing cow's milk (brand B control formulation) was significantly lower than all others tested, but had a high chroma value which indicated an increase in saturation. Incorporation of fermented peanut milk into formulations had a lightening effect, although differences in lightness were not significant in dressings based on the brand A mix. Significantly decreased *b* values in formulas containing fermented peanut milk indicated a significant decrease in yellowness.

The titratable acidity of salad dressings containing brand B mix and fermented peanut milk was significantly higher than that of the brand B control dressing. The opposite effect occurred in brand A formulations. A significant decrease in pH corresponded with the increase in titratable acidity of brand B formulations. The pH of brand A buttermilk (control) and fermented peanut milk formulas was not significantly different, suggesting a difference in buffering capacity of various brand A and B formulas.

Separation of emulsions prepared using brand A mix was detected within 4 days of storage at 5°C (Table 3). The amount of fluid released was greater in emulsions containing fermented peanut milk compared to the buttermilk control. Thus, the breaking of emulsions within this relatively short time could be considered as a problem since shelf life longer than 4 days would normally be desirable. Emulsions prepared using brand B dressing mix were quite stable over the 21-day storage period, thus reflecting a more effective performance of the stabilizing system compared to that in brand A formulas. The presence of buttermilk solids in brand B mix could also have aided emulsion stability.

In a separate series of experiments using brand C dressing mix, the effect of various ratios of buttermilk and fermented peanut milk on sensory, physical and chemical properties was determined. Results of sensory analyses by a trained panel are summarized in Table 4. Whiteness increased whereas creamy flavor and consistency decreased significantly as the substitution level of fermented peanut milk was increased. Creamy flavor is a very desirable attribute of ranch style dressing. This sensory quality clearly was adversely affected by substituting fermented peanut milk for buttermilk at a level of 50% or more in brand C dressing.

A significant decrease in consistency occurred in dressings in which buttermilk was substituted with 50% or more fermented peanut milk. Smoothness, however, appeared to be not greatly affected by amount of fermented peanut milk in the formulation. Such textural characteristics are very important in terms of quality since they can also affect flavor release and pourability. For these reasons, it would be advisable not to exceed a fermented peanut milk substitution level of about 25%, particularly when using the brand C mix. Lightness (L) of dressings decreased substantially as amount of fermented peanut milk in the formula was increased (Table 5). This was contrary to results from subjective evaluation of lightness (Table 4). However, chroma and hue angle of dressings were not greatly influenced by substituting fermented peanut milk for buttermilk.

Table 3—Emulsion stability of ranch-style salad dressing containing buttermilk, milk and fermented peanut milk when stored at 5°C up to 21 days

	mL of fluid separated from emulsion phase					
	Brand A			Brand B		
		Fermented peanut milk[a]			Fermented peanut milk[a]	
Days of Storage	Buttermilk	9085	14128	Milk	9085	14128
2	0	0	0	0	0	0
4	0.5	2.0	2.5	0	0	0
6	0.5	3.0	3.0	0	0	0
8	0.5	4.5	4.5	0	0	0
10	0.5	5.5	5.5	0	0	0
12	0.5	6.5	6.5	0	0	0
14	0.5	7.5	7.0	0	0	0
21	1.5	9.5	9.0	0	0.5	0.5

[a] Numbers (9085 and 14128) indicate sources of lactic bacterial cultures.

Substitution of fermented peanut milk at a level of 25% or more resulted in significant decrease in emulsion capacity (Table 5). Crenwelge et al. (1974) observed that comparing emulsion capacities of various materials was quite difficult and differences in results may occur due to the technique rather than to true differences in functional properties. The action of proteins as emulsifiers is influenced by concentration, speed of mixing, type of oil and type of emulsification system. In our study, differences in amount of oil required to reach the emulsion breakpoint of various formulations were relatively great. However, the oil emulsion capacity decreased as the substitution level of fermented peanut milk was increased. The pH and titratable acidity of salad dressing formulations containing various amounts of buttermilk and fermented peanut milk were not greatly affected except in the formulation containin no buttermilk. The slightly lower titratable acidity of fermented peanut milk (0.35 %) compared to buttermilk (1.0 %) caused this difference.

Brand C salad dressing containing various percentages of buttermilk and fermented peanut milk was subjected to emulsion stability tests at 5°C. No separation of fluid was detected within 21 days of storage in formulations containing 100% buttermilk or 75% buttermilk plus 25% fermented peanut milk.

Table 4—Intensity scores for ranch style salad dressing (brand C) containing various percentages of buttermilk and fermented peanut milk as judged by a trained panel

Sensory attribute	Peanut milk fermented with culture 9085[b]					Peanut milk fermented with culture 14128[b]				
	100/0[a]	75/25	50/50	25/75	0/100	100/0	75/25	50/50	25/75	0/100
Color										
Whiteness	5.5 d[b]	6.2 cd	7.1 bc	7.7 b	9.2 a	6.3 cd	6.5 cd	7.7 b	8.1 ab	9.1 a
Flavor										
Creamy	5.5 a	4.9 ab	3.3 bcd	2.6 cd	1.3 d	5.4 a	5.2 ab	3.1 bcd	2.6 cd	1.1 d
Beany/Green	2.8	2.2	2.3	2.3	2.0	3.3	3.2	3.4	3.2	2.5
Spicy	7.6 a	7.0 a	6.2 a	6.3 a	3.8 b	7.5 a	7.5 a	6.7 a	6.7 a	3.9 b
Sour	6.2 a	6.0 a	5.1 a	6.0 a	3.0 b	5.9 a	6.1 a	6.1 a	6.0 a	2.6 b
Sweet	2.3 ab	1.8 ab	2.2 ab	1.7 ab	0.9 b	2.6 a	2.1 ab	2.3 ab	2.1 ab	1.5 ab
Astringent	2.2	2.2	2.3	2.0	1.4	2.3	2.4	2.1	2.1	1.4
Mouthfeel										
Chalky	1.5	1.6	1.9	1.6	1.3	1.9	2.0	1.6	2.2	1.3
Consistency	9.0 a	8.2 a	5.4 b	2.9 d	0.9 e	8.7 a	7.7 a	4.9 bc	3.4 cd	1.2 e
Gummy	1.2	1.4	1.1	0.9	0.8	1.4	1.3	0.9	0.8	0.7
Smoothness	3.0 ab	2.9 ab	2.6 ab	3.0 ab	1.7 ab	2.8 ab	3.2 a	2.7 ab	2.8 ab	1.4 b

[a] Percentages of buttermilk/fermented peanut milk.
[b] Mean values in the same row which are not followed by the same letter are significantly different ($P \leq 0.05$).

Table 5—Physical and chemical measurements for ranch style salad dressing (brand C) containing various percentages of buttermilk and fermented peanut milk

Measurement	Peanut milk fermented with culture 9085					Peanut milk fermented with culture 14128				
	100/0[a]	75/25	50/50	25/75	0/100	100/0	75/25	50/50	25/75	0/100
Consistency (cps)	810 a[b]	730 a	405 b	–[c]	–	740 a	670 a	310 b	–	–
Color										
L	70.65 a	68.62 ab	63.15 bc	58.71 c	48.57 d	72.29 a	70.40 a	63.01 bc	58.79 c	46.82 d
a	−5.87	−6.07	−6.69	−5.60	−5.43	−6.74	−5.31	−6.05	−5.71	−4.66
b	17.32 ab	19.41 a	19.74 a	18.72 ab	17.78 ab	16.70 ab	16.83 ab	18.68 ab	18.01 ab	15.73 b
Chroma	18.33 abc	20.35 ab	20.84 a	19.54 abc	18.59 abc	18.11 abc	17.66 bc	19.66 ab	18.91 abc	16.40 c
Hue	108.87	107.31	108.71	106.60	106.97	111.81	107.43	108.15	107.62	106.62
Emulsion capacity[d]	128 a	116 b	108 cd	105 cd	102 cd	125 a	110 bc	107 cd	105 cd	101 d
pH	4.61 a	4.51 ab	4.46 ab	4.48 ab	4.37 b	4.58 a	4.48 ab	4.46 ab	4.49 ab	4.37 b
Titratable acidity (%)	0.78 ab	0.77 abcd	0.77 abcd	0.76 bcd	0.76 bcd	0.78 a	0.77 abc	0.77 abc	0.77 abc	0.75 d

[a] Percentages of buttermilk/fermented peanut milk.
[b] Mean values in the same row which are not followed by the same letter are significantly different ($P \leq 0.05$).
[c] Values were out of range when the same spindle number and rpm were used.
[d] Expressed as mL oil/159g base formulation.

Table 6—Emulsion stability of ranch style salad dressing (brand C) containing various percentages of buttermilk and fermented peanut milk which was stored at 5°C up to 21 days

Days of storage	mL of fluid separated from emulsion phase					
	Peanut milk fermented with culture 9085			Peanut milk fermented with culture 14128		
	50/50[a]	25/75	0/100	50/50	25/75	0/100
2	1.0	1.5	2.5	0.5	2.0	2.0
4	2.0	3.0	6.0	0.5	5.5	5.5
6	2.5	4.0	8.0	1.0	8.0	9.5
8	2.5	4.0	10.0	1.0	9.0	11.0
10	3.0	4.5	10.0	1.0	9.5	11.0
12	3.0	5.0	10.0	2.0	10.5	11.5
14	3.5	6.0	10.0	2.0	11.5	11.5
21	4.0	6.5	10.0	3.0	11.5	11.5

[a] Percentages of buttermilk/fermented peanut milk. No fluid separation occurred in 100/0 and 75/25 ratio formulas.

Substitution of buttermilk with fermented peanut milk at a 50% level or more caused separation of fluid from emulsions within 2 days storage (Table 6). Stability was greater in emulsions containing peanut milk fermented with the 9085 culture compared to emulsions containing peanut milk fermented with the 14128 culture. This was in agreement with observations on emulsion stability of dressings containing brand A and brand B mixes (Table 3). The marked decrease in consistency as determined instrumentally (Table 5) may correlate with the decrease in emulsion fluid-holding capacity.

CONCLUSIONS

INCORPORATION of fermented peanut milk into ranch style salad dressings prepared from three commercial brands of mixes resulted in decreased lightness (L, determined objectively), creamy flavor, oil emulsion capacity and consistency. Decreased consistency as determined instrumentally appeared to be correlated with low sensory scores for creamy flavor. The three brands of dressing mixes had different chemical, functional and sensory qualities of products produced from them. This was expected and indicated additional modifications to successfully facilitate incorporation of fermented peanut milk into ranch-style salad dressing would appear to have greater potential. Ranch-style salad dressing with acceptable quality can be made using fermented peanut milk substituted for buttermilk at a level up to 25%.

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Modification of Fruit Flavors by Aspartame and Sucrose

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ABSTRACT

Power functions for fruitiness intensity of fruit flavored (orange and strawberry) solutions unsweetened and sweetened with equi-sweet concentrations of aspartame and sucrose were developed using nine experienced panel members to assess any modification of fruit flavor by sweeteners. Enhancement (at low flavor levels) of fruitiness was observed in the aspartame sweetened systems even though the power functions of both flavorants were lowered by the addition of aspartame. The enhancement was more pronounced in the orange flavored system, suggesting a flavorant effect.

Key Words: fruit, flavors, aspartame, sucrose, sweeteners, flavorants

INTRODUCTION

DURING the last decade, the popularity of aspartame (APM) as an alternative to carbohydrate and other high potency sweeteners has increased. This has prompted many studies focusing on sensory properties of aspartame in simple and complex systems. Several references have been made to aspartame's ability to enhance or modify flavors, particularly fruit flavors (Beck, 1974; Cloninger and Baldwin, 1970; Homler, 1984; McCormick, 1975; Ripper et al., 1986). The earliest references to this observance were made in connection with chewing gum application patents. In those patents, Bahoshy et al. (1976) reported that aspartame produced longer-lasting sweetness and flavor and Bahoshy et al. (1977) found fruit flavors such as orange, lemon and grapefruit were enhanced and extended by aspartame.

Baldwin and Korschgen (1979) found that orange and cherry fruit flavors were more intense when sweetened with APM than with sucrose. However, they found no significant differences between APM or sucrose sweetened strawberry flavored beverages or the three flavored gelatin systems. It was observed from a time-intensity study by Larson-Powers and Pangborn (1978) that the maximum fruit flavor was higher in APM sweetened vs other sweetened lemon and strawberry flavored model systems. The results of Larson-Powers and Pangborn (1978) did not agree with Baldwin and Korschgen (1979) for the strawberry system. However, it is not known how the time intensity relationship of maximum intensity reported in the Larson-Powers and Pangborn (1978) study would be expected to relate to the intensity measurements in the Baldwin and Korschgen (1979) study.

Psychophysical relationships that relate physical concentration of a stimulus to sensory perception in the form of power functions were used in some of the earliest work on relative sweetness (Stone and Oliver, 1969; Moskowitz, 1970a, 1970b). Changes in the slope or elevation of these power functions were good indices of how an attribute was affected by other components in the system. Frank and Archambo (1986) used power functions as a baseline of sensory perception and then determined how the addition of another stimulus over the concentration range would affect intensity ratings. Schiffman (1984) also developed power functions to compare taste properties of aspartame to other sweeteners. Orange and strawberry flavors were chosen in our study to help determine if citrus flavors vs other flavors were affected differently by the sweeteners. Because Baldwin and Korschgen (1979) found no enhancement with strawberry flavors as opposed to Larson-Powers and Pangborn (1978) whose work showed an enhancement of flavor by aspartame, our study had the objective of clarifying the issue. To further investigate and quantify the enhancement capability of aspartame in comparison with sucrose, the objectives of our study were to develop sweetness power functions for sucrose and APM in spring water allowing equi-sweetness determination and then to develop fruitiness power functions for orange and strawberry flavored spring water unsweetened and sweetened with two equi-sweet levels of APM and sucrose. From these psychophysical relationships, it would be possible to quantify the modification of each flavor when combined with either APM or sucrose.

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MATERIALS & METHODS

Sample preparation

The following materials were used: aspartame (APM) from The NutraSweet Company (No. AD0120, GD. Searle Food Resources Inc., Skokie, IL), sucrose (My-Te-Fine from Fred Meyer, Inc., Portland, OR), natural orange flavor WONF (No. 24627, Food Materials Inc., Chicago, IL), natural strawberry flavor WONF (No 9157L, Borden Industrial Food Products, Columbus, OH), and spring water (5 gallon carboys, Aqua Cool, Salem, OR).

Solutions were prepared 24 hr in advance, stored at 5°C and brought to ambient temperature (22°C) prior to serving. Solutions were prepared in spring water and concentrations for each stimulus are presented in Table 1. The concentration range for each stimulus was based on a reasonable usage level of sweetener or flavorant in this system. Orange and strawberry flavorants were also prepared with equi-sweet levels of APM (0.04 and 0.093% w/v) and sucrose (5.0 and 10.0% w/v). Equi-sweetness was determined by use of the sweetness power function for each sweetener. References of sucrose (8.5% w/v) for sweetness intensity and unsweetened orange (0.95% w/v) or strawberry (1.2% w/v) flavorants for fruitiness intensity were available at all times.

Sensory method

Magnitude estimation was used by panelists to rate solution intensities. Panelists were instructed to taste the reference sample and assign its intensity a value of 50. After rinsing, they tasted the first sample and assigned it any value (except 0) corresponding to its ratio of

Table 1—Concentrations of sweeteners and fruit flavorants in single and binary solutions

Attribute rated[a]	Stimuli	Stimuli concentrations[b] 1	2	3	4	5
Sweetness	APM	0.03	0.041	0.056	0.07	0.10
	Sucrose	3.00	5.00	8.50	11.20	15.00
Fruitiness[c]	Orange	0.40	0.60	0.90	1.30	2.00
	Strawberry	0.60	0.90	1.20	1.70	2.50

[a] With a reference of sucrose (8.5% w/v) for sweetness and unsweetened orange (0.90% v/v) or strawberry (1.20% v/v) flavorants for fruitiness.

[b] All sucrose and APM concentrations were % w/v and flavorant (orange, strawberry) concentrations were % v/v.

[c] Also combined with equi-sweetness concentrations of aspartame and sucrose at 0.04, 0.093% w/v and 5.00, 10.00% w/v, respectively.

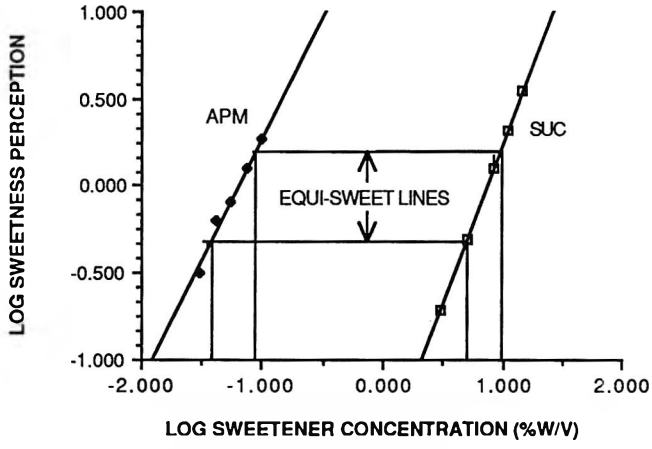

Fig. 1—Power functions of sweetness in sucrose (SUC) and aspartame (APM) sweetened spring water.

Table 2—Parameters from regression analysis of sweetness perception versus sweetener concentration and of fruitiness perception versus flavorant concentration for sweetened and unsweetened orange and strawberry solutions

Sweetener added to flavorant[x]	Y-Intercept[y]	Slope (n)	Coefficient of detm (r^2)	p-value
		Sweetener		
Sucrose	−1.56	1.79[a]	0.99	0.000
APM	+1.67	1.39[b]	0.98	0.001
		Orange flavorant		
none	−0.021	0.59[a]	0.98	0.000
sucrose(5.00)	−0.035	0.46[a]	0.92	0.010
sucrose(10.00)	+0.019	0.48[a]	0.94	0.006
APM(0.04)	+0.119	0.20[b]	0.72	0.059
APM(0.093)	+0.076	0.21[b]	0.74	0.052
		Strawberry flavorant		
none	−0.134	1.08a	0.97	0.003
sucrose(5.00)	−0.115	0.72[a]	0.87	0.021
sucrose(10.00)	−0.105	1.20[a]	0.98	0.002
APM(0.04)	−0.084	0.77[a]	0.95	0.005
APM(0.093)	+0.032	0.55[b]	0.90	0.014

[x] All sweetener concentrations are % w/v.

[y] Y-intercept expressed in log values.

[a,b] Slopes with the same letter are not significantly ($p \leq 0.05$) different.

sweetness or fruitiness intensity to the reference. For example, a sweetness intensity three times that of the reference was assigned a value of 150. All stimuli in the session were rated in the same manner and panelists rested 15–20 min between sessions. When fruitiness intensity was rated, panelists were instructed to ignore sweetness and concentrate only on the fruit flavor perceived.

Panelists

Nine students and staff from Oregon State University's Food Science & Technology Department were selected on the basis of experience with magnitude estimation for intensity ratings of solutions as well as motivation. Eight females and one male participated in an orientation session involving reviews of magnitude estimation, testing times and length of the study. During this session a series of sucrose solutions were judged by panelists and results were discussed in order to answer questions about procedure.

Environment

Testing took place in the Sensory Science Laboratory at Oregon State University. Samples were evaluated in individually partitioned booths, with red lights to mask color differences. The series of five solutions (20 mL) were served in 85 mL plastic cups, with an unlimited amount of the corresponding reference for each assessment.

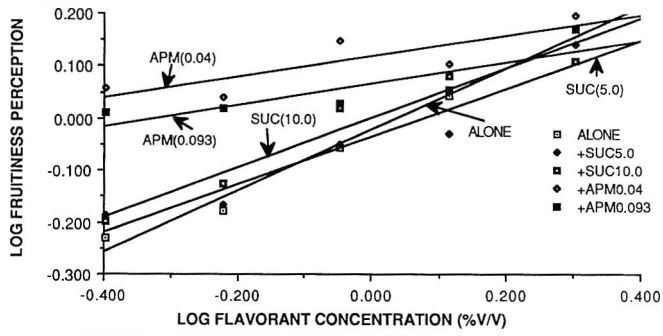

Fig. 2—Power functions of fruitiness in orange flavored spring water sweetened and unsweetened with two levels of sucrose (SUC) and aspartame (APM). Concentrations for sweeteners are expressed in % w/v.

Design

A randomized complete block design was used for each stimuli series (sucrose or aspartame and orange or strawberry flavor unsweetened and sweetened with sucrose or aspartame). The five-concentration series was presented in random order to each panelist along with the corresponding reference for evaluation at each session. Aspartame and sucrose sweetened solutions were evaluated on separate days and orange or strawberry flavored systems were tested on different weeks to facilitate panelist concentration on the specific fruit flavor. Two reps were completed.

Statistical analysis

In all experiments, magnitude estimates were normalized by geometric mean normalization (McDaniel and Sawyer, 1981). A three-way ANOVA was used to compare panelist, treatment, replication and panelist by treatment interaction effects for each experiment. Individual panelist regressions were adjusted by the least squares method to the best fitting line and slopes were compared by multiple regression to determine if differences were significant. Combined data regressions for each solution were adjusted by the least squares method to the best fitting line; then slopes and elevations for each experiment were compared by multiple regression.

RESULTS & DISCUSSION

Sweetness power functions

A three way ANOVA determined no significant differences ($p > 0.05$) in panelist or replication main effects and significance ($p \leq 0.05$) in the treatment main effect as well as panelist by treatment interaction. The significant interaction was due to a magnitude difference in panelists' ratings of solution intensities. All data were combined to construct the power functions presented in Fig. 1. Table 2 contains the intercept, slope, and coefficient of determination (r^2) for each power function. The slopes for both power functions were higher than other published values (Moskowitz, 1970a, 1970b, 1971); however, the sucrose slope was appreciably higher than the aspartame slope, which agreed with previous research. This difference in slope value could have been due to the concentration range of the sweeteners used, the panelists themselves or testing conditions. Two sweetness levels were selected by drawing two lines intersecting both power functions (Fig. 1), resulting in two equi-sweetness levels for each sweetener. These levels were not tested formally for equi-sweetness in the system with added orange or strawberry flavor.

Orange fruitiness power functions

Results for the regression analysis of orange flavor power functions are presented in Table 2 and graphically represented in Fig. 2. The linear model was significant ($p \leq 0.05$) for all formulations except the 0.040% and 0.093% w/v aspartame sweetened solutions. However, the linear model fit both levels

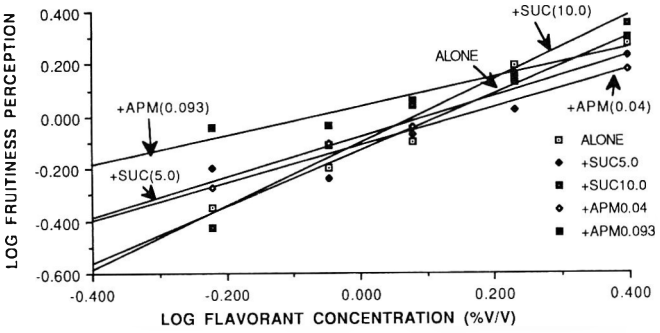

Fig. 3—Power functions of fruitiness in strawberry flavored spring water sweetened and unsweetened with two levels of sucrose (SUC) and aspartame (APM). Concentrations for sweeteners are expressed in % w/v.

of the APM sweetened systems data better than other models. The slopes for both aspartame sweetened systems were very low (0.20, 0.21) and there was no significant difference in fruitiness rating among data points across flavorant levels. Multiple regression was conducted to test differences in slope and elevation caused by addition of both sweeteners. The slope of the unsweetened flavorant was not significantly lowered by either level of sucrose but was lowered ($p \leq 0.05$) by both levels of aspartame. No significant difference in fruitiness was found for either system sweetened with sucrose as compared to the flavorant alone, but fruitiness of both systems sweetened with aspartame was significantly enhanced below the 0.040% and 0.06% v/v flavorant concentration. The difference in magnitude and growth of fruitiness perception is easily seen in Fig. 2 where sweetening with aspartame greatly enhanced initial fruitiness by as much as 100%. Fruitiness intensity of APM and sucrose sweetened solutions were not significantly different at higher flavor levels due to the low slopes of fruitiness power functions for APM sweetened systems.

Strawberry fruitiness power functions

Results for the regression analysis of strawberry flavored power functions are presented in Table 2 and graphically represented in Fig. 3. All systems were significantly ($p \leq 0.05$) described by a linear model. Multiple regression resulted in no significant differences in slopes of fruitiness power functions except at the 0.093% APM sweetened system which was different ($p \leq 0.05$) from all other slopes. The magnitude of fruitiness perception was increased by the lower level of sucrose and both levels of aspartame but was significant only for the 0.093% aspartame sweetened system at 0.60 and 0.90% v/v flavorant concentrations. The differences in fruitiness modification is evident in Fig. 3 where the greatest increase in initial fruitiness perception is produced by the higher level of aspartame. As with the orange-flavored system, APM sweetened fruitiness power functions had lower slopes than the sucrose or unsweetened systems; therefore, differences in fruitiness intensity were not significantly different among higher flavorant levels. The modification of slope and elevation was less in the strawberry-flavored system than in the orange-flavored system, which indicated a possible flavorant difference.

Key to this study was the determination of equi-sweetness without any influence from the fruit flavor; therefore, equi-sweetness was determined based on taste only. Subsequent addition of flavorant might influence the previous sweetness rating as well as adding fruitiness. In addition, the rating of fruitiness might be influenced by any additional sweetness perception. One possible explanation for the enhancement of fruitiness by aspartame is the mutual enhancement of sweetness and fruitiness in the sweetened and flavored system. This could only be tested by setting equi-sweetness levels prior to adding flavorant.

The fruitiness slopes of the aspartame sweetened systems were flat, but all points were at a high fruitiness level. Therefore, even at the lowest flavorant levels for aspartame sweetened systems, the fruitiness was as high as the highest flavorant level samples for the sucrose sweetened systems. Again, this may have been the result of mutual enhancement of fruitiness and sweetness, although only fruitiness was measured.

Our results provide useful information for future investigations as well as possible applications in the food industry. The orange and strawberry flavorants were modified by sucrose and aspartame. As in the Larson-Powers and Pangborn (1978) and the Baldwin and Korschgen (1979) studies, the enhancement effect appeared stronger in orange than in the strawberry flavored systems. This may have been due to the fact that there was more of a retronasal sweetness aromatic component in orange flavor than in strawberry flavor. More sweetness aromatics in the orange flavored system would account for the larger effect of sweetening with aspartame than observed in the strawberry system. This lower level of sweetness aromatics in the strawberry flavored systems could also account for the lack of enhancement by aspartame on strawberry flavor reported by Baldwin and Korschgen (1979). Aspartame sweetened solutions showed as much as 100% increased fruitiness perception from 0.40 to 0.90% flavorant level in the orange system and from 0.60 to 1.20% flavorant level in the strawberry flavored system. Sucrose sweetened solutions did not show this enhancement of either orange or strawberry fruitiness.

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Accelerated Kinetic Study of Aspartame Degradation in the Neutral pH Range

MENEXIA N. TSOUBELI and THEODORE P. LABUZA

ABSTRACT

The degradation of aspartame in solution as a function of temperature (70–100°C), buffer concentration (0.01–0.1M phosphate), and pH (6–7) was studied in order to estimate losses during thermal processing and storage of aseptic milk-based drinks. Prior data have been mostly on acid carbonated beverages. First order rate constants were obtained in all the conditions with activation energies in the range 14–20 kcal/mole. An increase in both pH and buffer concentration caused an increase in rate of loss. These data were used to predict losses that would occur during pasteurization and sterilization conditions. Experiments at 4 and 30°C showed significant losses would occur during 4 and 30°C temperature storage and extrapolation from high temperatures predicted faster degradation rates than those found.

Key words: pH, degradation, kinetics, accelerated storage

INTRODUCTION

THE DIPEPTIDE sweetener, aspartame (N-L-α-aspartyl-L-phenylalanine-1-methyl ester, α-APM), is regulated by the FDA as a flavor enhancer and nutritive sweetening agent. Aspartame is about 180 times sweeter than sucrose (Homler, 1984), and has been approved for use in many applications such as dry foods, carbonated beverages and refrigerated flavored milk beverages (FDA, 1988). Aspartame has limited stability in aqueous solutions (Mazur, 1976; Schertz et al., 1983; Homler, 1984; Prudel et al., 1986; Stamp, 1990) as well as in intermediate moisture systems (Bell, 1989; Bell and Labuza, 1991b). Its stability is affected significantly by both pH and temperature of the system, and decomposition follows pseudo-first order kinetics. The maximum stability occurs in the pH range 3 to 5 (Prudel et al., 1986; Stamp, 1990).

A variety of methods have been used to detect and identify aspartame and its decomposition products including thin layer chromatography (Daniels et al., 1984; Sherman et al., 1985; Ozol, 1987). High Performance Liquid Chromatography (Schertz et al., 1983; Prudel et al., 1986; Stamp and Labuza, 1989b) and Fourier Transform InfraRed spectrometry (Chess and Gerson, 1986). Aspartame decomposition follows acid-base catalysis in solution with formation of 3,6-dioxo-5-phenylmethyl-2-piperazineatic acid (DKP), the major decomposition product (Furda et al., 1975). This is produced by intramolecular cyclization of aspartame with loss of methanol whereas α-L-aspartyl-L-phenylalanine (α-AP) forms by base catalyzed hydrolysis of the methyl ester with loss of methanol (Stamp and Labuza, 1989a). These are the only products produced in the basic pH range. At acid pH other products such as L-phenylalanine-1-methyl ester, L-aspartic acid and L-phenylalanine (Schertz et. al., 1983; Prudel et al, 1986; Stamp and Labuza, 1989a) as well as the structural isomer of aspartame, β-APM (Stamp and Labuza, 1989a) were also produced. All of the decomposition products are non-sweet so that decomposition leads to loss of initial sweetness (Homler, 1984).

Since the approval of aspartame for use in refrigerated, flavored milk beverages, the study of the decomposition kinetics of aspartame in products like aseptic dairy drinks is of interest.

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The objective of our study was to systematically obtain kinetic data for aspartame degradation under higher (accelerated) temperature conditions (70–100°C), as a function of pH (6.0, 6.5, 7.0), and buffer concentration (0.01–0.1 M/L phosphate buffer). Knowledge of the reaction rate constants and activation energies under those conditions could then be used to predict losses occurring during both processing and storage of milk-based drinks. Degradation of aspartame was also studied at 4 and 30°C as a function of buffer concentration of pH 7.0, and results were compared to shelf life predicted from higher temperature studies.

MATERIALS & METHODS

α-L-ASPARTYL-L-PHENYLALANINE-1-METHYL ester (α-APM) and 3,6-dioxo-5-phenyl-methyl-2- piperazineacetic acid were obtained from the NutraSweet Co. (Skokie, IL). α-L-aspartyl-L-phenylalanine (α-AP) was obtained from Sigma Chemical Co. HPLC grade acetonitrile and monosodium and disodium phosphate were obtained from Fisher Scientific Co. (Fair Lawn, NJ). Organic free water was produced by a Milli-Q purification system (Millipore, Bedford, MA). HPLC grade 85% phosphoric acid was obtained from Fisher (Fair Lawn, NJ). HPLC grade sodium salts of 1-heptanesulfonic acid were obtained from Eastman Kodak Co. (Rochester, NY).

The HPLC method of Stamp and Labuza (1989b) was used. The column was an analytical NovaPak C_{18} (15 cm × 3.9 mm; Waters Associates, Natick, MA) containing an "endcapped" 4 mm spherical stationary phase. The HPLC system used to isolate the aspartame decomposition products consisted of a CP8810 precision isocratic pump from Spectra-Physics, a 757 absorbance detector and a Hewlett Packard 3396A integrator. The aqueous component of the mobile phase was composed of equal 5 mM amounts of 1-heptanesulfonic acid and monosodium phosphate in 1 L of organic free, HPLC grade water. This was then mixed with HPLC grade acetonitrile in the ratio of 20:80 (v/v%). The pH of the solution was adjusted to 3.0 with HPLC grade 85% phosphoric acid. A flow rate of 1.0 mL min^{-1} was used, with detection by UV absorbance at 214 nm.

Aqueous solutions of aspartame consisted of mono and disodium phosphate buffer and 2 mM (600 ppm) aspartame. The buffer concentration was 0.01M/L, 0.05M/L or 0.1 M/L and at a pH of 6.0, 6.5 or 7.0 for 0.01M/L and 0.1M/L and 6.5 or 7.0 for 0.05M/L. Temperature ranged from 70 to 100 °C for all conditions. Aliquots (2 mL) of each aspartame solution were sealed into 2 mL glass ampules and placed in a (HAAKE E52) circulating temperature-controlled oil bath (± 0.01 °C). At predetermined time intervals, samples were removed and immediately cooled for 2 min in an ice water bath. After opening the ampules, an aliquot (1 mL) was diluted with 5 mL of monosodium phosphate at pH 3 and then stored at 4 °C to inhibit further decomposition until analysis could be completed. HPLC standards at four concentrations were made and evaluated each day of analysis. Based on the procedures of Labuza (1984), at least six data points over two half lives (75% loss) were collected and the 95% confidence limits and r^2 values were calculated by linear regression of the proper concentration function vs. time. If the 95% confidence limits of a given slope (k-rate constant for ln(amount) vs time; or E_A-lnk vs 1/T) did not overlap that of another slope for a different condition, then the two slopes were different ($p > 0.05$). Generally, because of analytical capacity, more data points were collected under conditions where the reaction was slower.

For the 4 and 30°C temperature studies, the buffer concentration was either 0.1 M/L or 0.01M/L at pH of 7. At a given temperature and buffer concentration that is the most severe condition where aspartame degrates fastest. Therefore it was used as an indicator of the

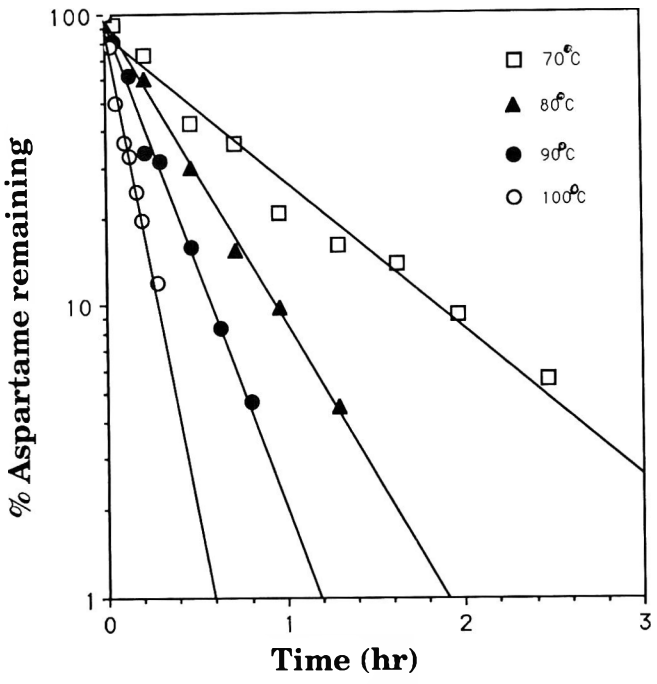

Fig. 1—Degradation of aspartame as influenced by temperature at 0.01M buffer concentration and pH 7.

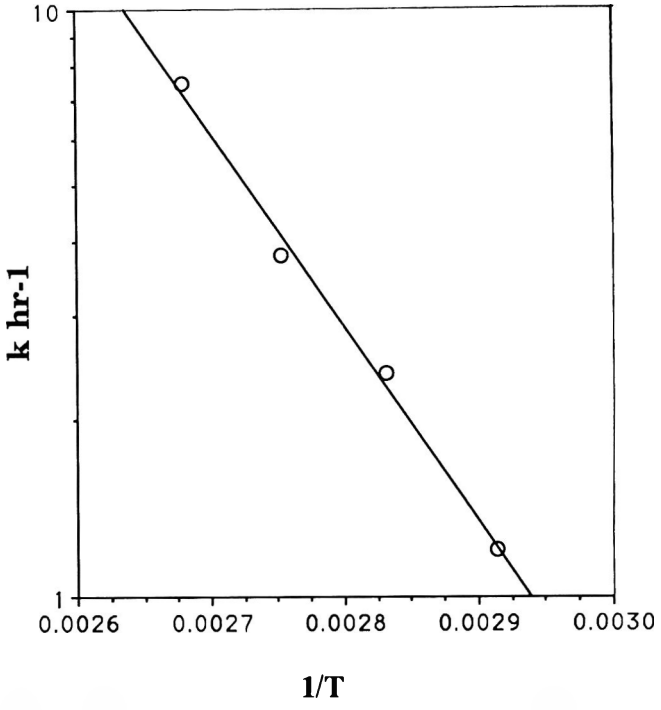

Fig. 2—Arrhenius plot for aspartame degradation at 0.01M and pH 7.

Table 1—Rate constants (±95% confidence limits) for aspartame degradation, effects of acidity and temperature[a]

System		Rate constants (hr^{-1})			
		70 °C	80 °C	90 °C	100 °C
M	pH	(r^2)	(r^2)	(r^2)	(r^2)
0.01	7.0	1.20 ± 0.20 (0.98)	2.40 ± 0.14 (0.99)	3.80 ± 0.32 (0.99)	7.50 ± 1.03 (0.98)
0.01	6.5	0.16 ± 0.04 (0.98)	0.36 ± 0.14 (0.96)	1.10 ± 0.01 (0.99)	1.40 ± 0.22 (0.96)
0.01	6.0	0.01 ± 0.002 (0.93)	0.03 ± 0.001 (0.93)	0.07 ± 0.01 (0.97)	0.15 ± 0.03 (0.94)
0.05	7.0	3.10 ± 0.20 (0.99)	4.80 ± 1.00 (0.95)	11.20 ± 2.90 (0.94)	20.70 ± 6.60 (0.91)
0.05	6.5	1.0 ± 0.05 (0.99)	2.10 ± 0.20 (0.99)	4.60 ± 0.90 (0.95)	8.90 ± 1.47 (0.97)
0.1	7.0	5.7 ± 0.90 (0.97)	9.30 ± 1.30 (0.98)	16.60 ± 5.6 (0.89)	28.60 ± 10.13 (0.96)
0.1	6.5	1.95 ± 0.08 (0.99)	4.20 ± 0.70 (0.97)	7.10 ± 1.00 (0.97)	12.20 ± 2.50 (0.95)
0.1	6.0	0.41 ± 0.02 (0.99)	1.04 ± 0.06 (0.99)	2.30 ± 0.12 (0.99)	5.90 ± 0.90 (0.97)

[a] All rates significantly different at $p > 0.05$ as a function of temperature increase, buffer concentration increase and pH decrease.

Table 2—Half life for aspartame degradation as affected by acidity and temperature with (rate increase factor as compared to lowest pH tested)

System		Half life (min)			
M	pH	70 °C	80 °C	90 °C	100 °C
0.01	7.0	35 (112)	17 (68)	11 (38)	5.5 (56)
0.01	6.5	208 (15.7)	115 (12)	38 (14.5)	30 (5)
0.01	6.0	4158	1386	594	278
0.05	7.0	13 (3.5)	9 (2.1)	3.7 (2.7)	2 (2.7)
0.05	6.5	42	20	9	5
0.1	7.0	7 (14.6)	5 (8.4)	2.5 (9)	1.5 (7)
0.1	6.5	22 (4.3)	10 (4.2)	6 (3.6)	3.4 (1.9)
0.1	6.0	104	40	18	7

least shelf life at 4 and 30°C. The same experimental procedure was followed, but instead of the oil bath, an incubator with temperature at either 4 or 30 ±0.5°C was used.

RESULTS & DISCUSSION

THE DEGRADATION of aspartame as a function of temperature at pH 7.0 in 0.01 M phosphate buffer is shown in Fig. 1. The decomposition of aspartame can be represented as a pseudo first order reaction (Bell and Labuza, 1991a,b; Prudel et al., 1986) by the equation:

$$\ln\{[APM]/[APM_0]\} = -k_{obs}\, t \qquad (1)$$

Thus a semilog plot of the percentage of aspartame remaining vs. time was a straight line with slope k_{obs}. Table 1 summarizes rate constants for all conditions employed. Table 2 shows the same results reported as half life, for easier comparison. As noted, except for pH 6.5 or less and 0.01M buffer concentration, the half life was generally less than 1 hr. At the same buffer concentration and temperature, aspartame had a greater rate of decomposition as pH increased, indicating base catalysis. At constant buffer and pH, there was a statistically significant higher rate of degradation at each higher temperature as expected from Arrhenius kinetics.

The temperature dependence of a system is best represented by the Arrhenius activation energy (E_a) at any given solution composition condition. Figure 2 shows an Arrhenius plot for 0.01M buffer concentration at pH 7. Table 3 shows the calculated E_a and Q_{10} values for each composition tested. At 0.01M and pH 7, the E_a was 15.40 ± 1.5 kcal/mol. This value was statistically similar (Labuza, 1984) to the value of 16.7 kcal/mol reported by Stamp (1990) for the same composition, which was calculated using data over the 50 to 100 °C range. It was also statistically equivalent to the value of 15.2 ± 0.8 kcal/mole found by Bell and Labuza (1991b) over the 25–37°C range for a system consisting of microcrystalline cellulose, water and aspartame at water activity very near 1 and pH 7.

As can be seen from Table 3, the activation energy increased significantly as pH decreased from 7 to pH 6, i.e. the reaction rate became more temperature sensitive as pH decreased. However differences were not significant if we consider only a 0.5 pH difference (e.g. between 6.5 and 7) as compared to 6 to

Table 3—Activation energies and Q_{10} (70 to 100 °C) for aspartame degradation in solution as affected by acidity (±95% confidence limits)

System			
M	pH	E_a (kcal/mole)	Q_{10}
0.01	7.0	15.4 ± 1.5[a]	1.80
0.01	6.5	18.8 ± 3.3[ab]	2.10
0.01	6.0	22.2 ± 2.8[b]	2.40
0.05	7.0	16.2 ± 3.4[a]	1.98
0.05	6.5	18.7 ± 1.8[a]	2.06
0.1	7.0	13.8 ± 2.2[a]	1.84
0.1	6.5	15.2 ± 2.1[a]	1.81
0.1	6.0	22.6 ± 1.2[b]	2.40

[a,b] Values with similar superscripts not significantly different at $p>0.05$ for the same buffer concentration or pH.

7. Bell and Labuza (1991b), found that at water activity 0.68 (moisture limited system), the activation energy at pH 3 was 23.5 ± 1.6 kcal/mol while at pH 7 in the same limited moisture system, it was 16.5 ± 1.3 kcal/mol. This suggested that the temperature sensitivity of the system depended on both the composition and water activity and that the E_A probably decreased above and below the isoelectric point of aspartame, pH 5.4. This is very common for acid base catalyzed degradation reactions as with certain pharmaceuticals (Conners et al. 1986).

Based on our results, both buffer concentration and pH are important in degradation of aspartame. Such degradation being both acid and base catalyzed, is expected to be most stable near the isoelectric point with the rate increasing at higher and lower pH. As seen in Table 1, the rate constant for degradation significantly increased as pH increased in the range 6 to 7 as expected from base catalysis. Similar results were obtained in solutions by Stamp (1990) and Homler (1984) and by Bell and Labuza (1991b) above pH 5, in intermediate moisture model systems.

Since aspartame degradation is an acid-base catalyzed reaction, the dependence of pH on rate of reaction, assuming three independent interactions of water and its ions, results in:

$$k_{obs} = k_o + k_{H^+}[H^+] + k_{OH^-}[OH^-] \quad (2)$$

If the ionization constant of the water is taken into consideration, which by definition is:

$$K_w = [H^+][OH^-] = 10^{-14}\ Mol^2\ L^2 \quad (3)$$

then k_{obs} is given by the following relationship:

$$k_{obs} = k_o + k_{H^+}[H^+] + k_{OH^-}\{K_w(1/\{[H^+]\} \quad (4)$$

This suggests that a plot of k_{obs} vs pH would produce a 'bell-shaped' curve with a minimum which corresponds to maximum stability of aspartame, a region below the isoelectric point which defines acid catalyzed reactions, k_{H^+}, and another region above the isoelectric point which defines base catalyzed reactions, k_{OH^-}. Thus in taking the logarithm of equation (4) for the acid region (pH < 5) and assuming that the base catalyzed and water catalyzed steps in the equation are negligible, the resulting equation gives a straight line with slope −1 because:

$$\log k_{obs} = \log k_{H^+} + \log [H^+] = c_1 - pH \quad (5)$$

Equation (5) indicates that a ten fold decrease in reaction rate for each decline by one pH unit should be expected. Conversely, for the basic region, assuming all other reactions in Eq. (4) are negligible except for base catalysis, then:

$$\log k_{bos} = \log k_{OH^-} + \log K_w - \log [H^+] = c + pH \quad (6)$$

and as pH increases by one unit above the isolectric point the rate increases ten fold. For a 1/2 unit pH change, given the above considerations, the rate increases by 3.2 times.

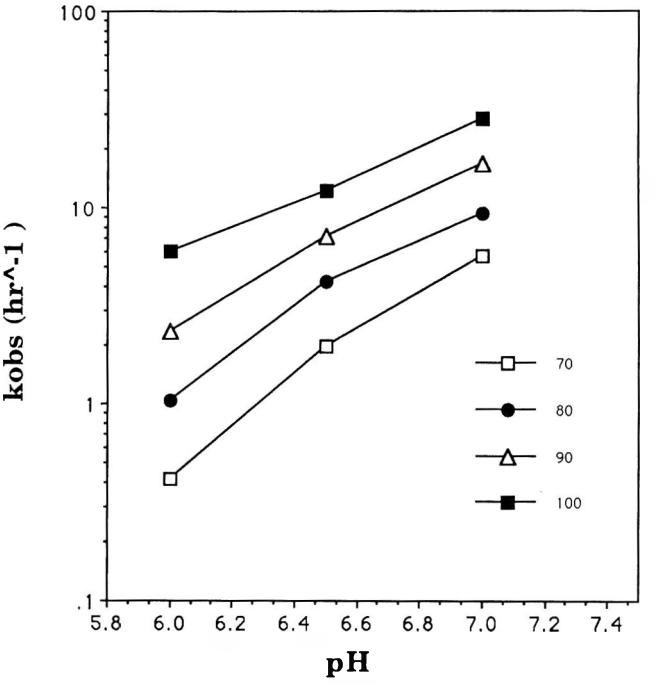

Fig. 3—Rate constant of aspartame degradation as a function of pH (basic range), at 0.1M phosphate buffer at different temperatures.

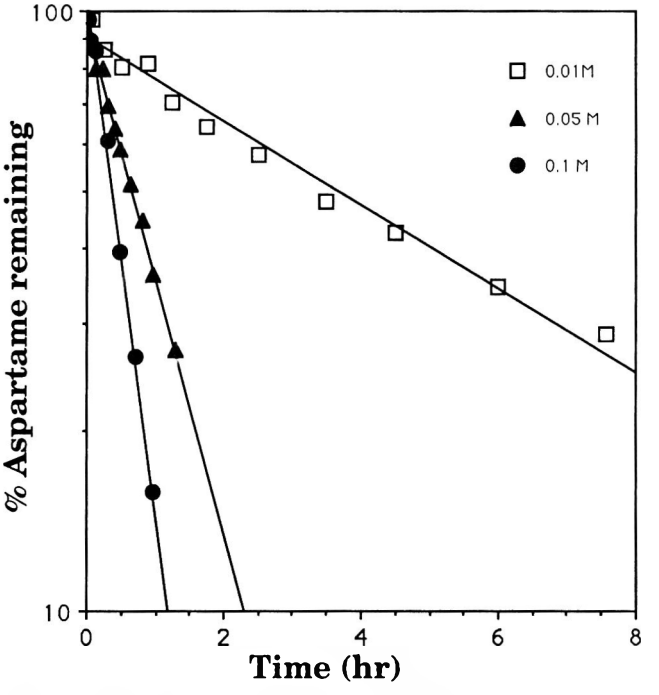

Fig. 4—Degradation of aspartame as influenced by phosphate buffer concentration at 70 °C, pH 6.5.

Figure 3 shows the effect of pH on the rate constant for the 0.1M buffer concentration at 70–100 °C, and Table 2 summarizes the increase in rate for a pH change from the lowest value tested (in parentheses). As seen in Table 2, changes in rate as a function of pH did not follow the theoretical predictions of equation (6) for the 0.01M buffer concentration but were the right magnitude for the 0.05 and 0.1M buffer concentrations. At low buffer concentration (0.01M), due to pH

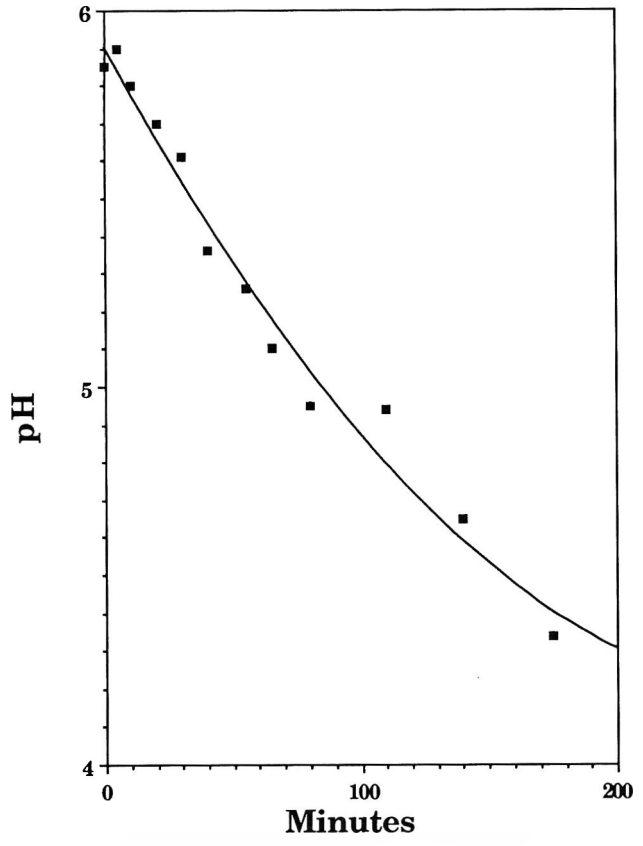

Fig. 5—Decrease in pH as function of time for the 0.01M phosphate buffer system, pH 6 and 110 °C.

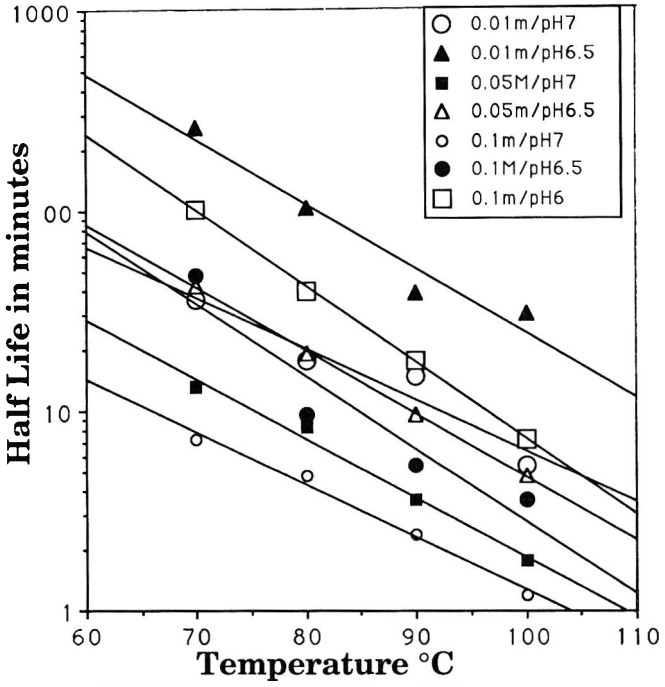

Fig. 6—Shelf life plot of aspartame stability of function of temperature for all systems studied.

lowering that occurred during a test as shown in Fig 5, Eq. (6) was not followed and the rate decreased significantly.

The buffer concentration effect for pH 6.5 and 70 °C is shown in Fig 4, which indicates that the rate increased as buffer concentration increased from 0.01M to 0.1M. This is defined as specific base cayalysis (Jencks, 1976). Similar results were

Table 4—Predicted aspartame loss under pasteurization and sterilization conditions at 0.1M phosphate buffer concentration, pH 7

Temperature (°C)	Time	Aspartame loss (%)
FDA pasteurization requirement		
63	30 min	83
72	15 sec	2.5
88	1 sec	0.4
96	0.05 sec	0.03
100	0.01 sec	0.0
UHT pasteurization		
138	>2 sec	8.8
UHT Sterilization		
140	6.5 sec	28
150	4 sec	26

obtained for all conditions examined (Table 1). For example, the half life of aspartame for pH 6.5 at 70 °C and 0.01M was 258 min, at 0.05M it was 42 min while at 0.1M it decreased to 24 min. Thus there was more than a tenfold increase in rate for a tenfold increase in buffer concentration. This occurred because the buffer ion directly participated in the reaction by donating a proton as well as by interacting with the charge on the amine group of aspartame. This stable, but presumably weak, complex (depending on type of anion), is shown in the following equations:

$$HB \rightleftarrows H^+ + B^- \quad (7)$$

$$APM + H^+ \rightarrow \text{degradation} \quad (8)$$

$$APM\text{-}NH_3^+ + B^- \rightarrow APM\text{-}NH_3^+ \cdot\cdot B^- \quad (9)$$

As the concentration of buffer increases, concentration of the $HPO_4=$ ions, (when phosphate buffer is used), increases. Thus the reaction of interchanging protons with the amine group of the aspartame molecule is enhanced leading to faster cyclization to DKP. The HPO_4 = ions are not consumed throughout the reaction, if the buffering capacity is high enough, and thus could be converted back to HB. At 0.01M buffer concentration and pH 6, the pH decreased throughout the reaction passing into the acid catalyzed region (Fig 5). This can be attributed to the limited buffering capacity at that buffer concentration and pH. Phosphate buffer in the pH range studied has a maximum buffering capacity at its second pK_a of 7.2, while its minimum buffering capacity is at pH 6 (Jencks, 1976). Thus at pH 6, one should expect minimal buffer catalysis; and since the buffer capacity is at its minimum, the pH would decline, decreasing the loss rate. At the same low buffer concentration, but at pH 6.5 or greater, the buffering capacity would be enough to prevent this pH decrease. In those cases, the pH always was within 0.1 pH unit of the initial value at the end of the experiment. Stamp (1990) found the same effect in solution experiments.

Figure 6 shows a semilog shelf life plot of half life vs. temperature based on the method of Labuza and Kamman (1983). From a comparison of half lives the stability of aspartame was almost the same for 0.01M, pH 7.00, 0.05M at pH 6.50 and 0.1M and pH 6.00. This shows the flexibility in formulation of food products to protect aspartame, since the same shelf life could be achieved with different compositions with variations in pH and buffer concentration. Extrapolation of the rate constant for both pasteurization and UHT sterilization conditions, as well as for refrigerated and 30°C temperature storage was done in order to predict loss of aspartame under thermal processing and typical storage conditions of aseptic dairy based beverages. Data for 0.1M, pH 7.00 were used which represents the most severe of all conditions.

Table 4 summarizes results from extrapolation to pasteurization and sterilization conditions. Pasteurization was based on FDA requirements for milk (21 CFR 131.3) and the sterilization conditions were typical for aseptic milk packaging.

Table 5—Predicted half lives for aspartame degradation in a system at 3.5 or 30°C

System		Half life (days)	$k_{obs}(d^{-1})$	Half life (days)	k_{obs}
M	pH[a]	3.5 °C	(r^2)	30 °C	(r^2)
0.01	7.0	6.0		0.5	
0.01	6.5	120		6.4	
0.01	6.0	6108*		188.5*	
0.01	7.0				
experimental value		32.1	0.022 ± 0.004 (0.97)	1.5	0.48 ± 0.09 (0.96)
0.05	7.0	3.6		0.3	
0.05	6.5	21.5		1.10	
0.1	7.0	0.8		0.1	
0.1	6.5	3.4		0.3	
0.1	6.0	204.4		5.6	
0.1	7.0				
experimental value		4.8	0.14 ± 0.03 (0.96)	0.1	5.1 ± 0.63 (0.99)

[a] pH decreases throughout the reaction

The loss of aspartame was negligible for pasteurization temperatures, other than the low temperature (63 °C) where, with the required 30 min processing time, it was 83%. This would require a significant overrun of aspartame to maintain sweetness. At sterilization conditions, the loss of aspartame was about 26–28% in the 4 to 6.5 sec period of processing, which is important. One must also take into consideration the time required for the system to reach the desired temperature (come-up time) as well as the time needed for cooling, which will depend very much on system design and would contribute to further aspartame loss. Our data along with time/temperature profiles could be used to adjust initial aspartame concentrations to account for prospective losses.

The estimate of shelf life of aseptic aspartame sweetened dairy beverages at refrigerated (3.5°C) and 30°C storage conditions was based on the definition that the end of shelf life was the loss of a 50% overun of aspartame concentration set above the "just acceptable" sweetness level. Table 5 summarizes predictions and true results for stability of aspartame at the two temperatures. As shown, the shelf life of aspartame was relatively short at pH 6 to 7 for room temperature storage. This indicated that without some type of protecting system, aspartame would not be stable in liquid dairy systems unless specific buffer and pH conditions compatable with the other ingredients could be used (e.g., pH 6 and 0.1M buffer for a refrigerated product). Thus the pH/buffer concentration relationship is very important in determining shelf life of products sweetened with aspartame.

In comparing true results from the low temperature study with those of the accelerated shelf life (ASLT) prediction (Table 5), several predictions were far too low. For instance, the predicted half life at 0.01M buffer, pH 7 was 6 days whereas the experiment gave a half life of 32 d, more than 5 times larger. This was also reported by Stamp (1990) who attributed the difference to lack of data at 3.5°C, which was not the case here. Those low predictions could be explained by the fact that aspartame degradation, at near neutral pH, proceeds in two parallel reactions with two separate rate constants with different activation energies, 16.6 for DKP formation and 20.0 kcal/mol for α-AP formation (Stamp, 1990). If activation energies for the individual reactions from these higher temperature studies differ and the rates were similar, then extrapolation to lower temperatures may not be valid due to a change of slope in the Arrhenius plot from high to low temperature regime. However at pH 6–7, the rate of DKP formation ranged from 10 to 20 times greater than α-AP formation over 100 to 30°C, thus some unknown factor may have caused the decreased rate at 3.5°C. Note that the predictions made for pasteurization conditions in Table 4 using data from the high temperatures regime study would not be expected to result in error, since the pasteurization temperature range overlaps the temperature range used in the experiments. In addition, prediction for aspartame loss under UHT pasteurization and sterilization conditions at (138–150°C) should not result in similar kinetic prediction errors since the crossover of Arrhenius lines for the two degradation steps based on the data of Stamp (1990) was greater than 200°C. Thus for the temperature range 138 to 150 °C, activation energies of the two degradation steps should not differ significantly and therefore the prediction would not be expected to result in much error.

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Water and Molecular Weight Effects on Glass Transitions in Amorphous Carbohydrates and Carbohydrate Solutions

YRJÖ ROOS and MARCUS KAREL

ABSTRACT

The effects of water, freeze-concentration and effective molecular weight (M_e) on glass transition (T_g) of maltose and maltodextrins were studied, and methods to predict T_g were used to establish state diagrams. T_g of maximally freeze-concentrated solutes (T_g') and onset of ice melting (T_m') increased with M_e, and for high molecular weight polysaccharides T_g' and T_m' were predicted to have the same temperature value. Ice formation at $T_g' < T < T_m'$ was time dependent. Unfrozen water in maximally freeze-concentrated matrices was about 20% independently of M_e. The state diagrams can be used to evaluate physical state of frozen and dehydrated foods.

Key Words: Carbohydrates, molecular weight, glass-transition, freeze-concentration, water

INTRODUCTION

DEHYDRATED and frozen food materials often exhibit phase transitions typical of amorphous polymers (e.g. Parks and Thomas, 1934; Kauzmann, 1948; White and Cakebread, 1966; Levine and Slade, 1986; Roos, 1987; Slade and Levine, 1991). In foods, glass transition and crystallization of amorphous components affect their physical state. Changes in physical state including crystallization are often controlled by glass transition temperature (T_g) above which they may occur time-dependently (Roos and Karel, 1990; Roos and Karel, 1991a). Most amorphous food components are miscible with water which acts as a plasticizer causing decreasing transition temperatures with increasing water content (e.g. Levine and Slade, 1986; Roos, 1987; Roos and Karel, 1990; Slade and Levine, 1991). Thus an increase in temperature or water content may cause changes in physical state which are observed as stickiness, collapse and crystallization in dehydrated foods (Tsourouflis et al., 1976; Downton et al., 1982; Roos, 1987; Roos and Karel, 1991a,b).

The T_g is specific to each anhydrous material, although it may slightly vary depending on thermal history and experimental conditions (e.g. Tant and Wilkes, 1981; Wunderlich, 1981). Water decreases the T_g which at high water contents theoretically approaches that of pure water (Luyet and Rasmussen, 1967; Franks et al., 1977; Levine and Slade, 1986). At freezing temperatures ice formation leads to separation of plasticizing water as ice thus leading to an increased solute concentration, increased T_g of the unfrozen matrix, and decreased melting point of ice (Luyet and Rasmussen, 1968; Rasmussen and Luyet, 1969; Bellows and King, 1973; Levine and Slade, 1986; 1988; Roos and Karel, 1991c,d). Ice formation in the unfrozen matrix may occur during rewarming of rapidly cooled materials (e.g. Luyet and Rasmussen, 1968; Simatos et al., 1975; Williams and Carnahan, 1989; Roos and Karel, 1991d; Slade and Levine, 1991) causing a concurrent increase of the effective T_g (Levine and Slade, 1986; Roos and Karel, 1991c,d). At maximum freeze-concentration glass transition (T_g') and onset of ice melting (T_m') occur at constant temperatures which are independent of initial solute concentration (Franks et al., 1977; Levine and Slade, 1986; Roos and Karel, 1991c,d). Theoretically T_g' and T_m' should coincide (Franks et al., 1977; Levine and Slade, 1986; Slade and Levine, 1991). However, an exact transition temperature is not typical of glass transitions which usually occur over a temperature range of 10 to 20°C, and the onset temperature of T_g' and T_m' may be different (Roos and Karel, 1991d).

Franks et al. (1977) proposed the use of state diagrams to characterize the physical state of amorphous materials as a function of concentration. In such diagrams T_g and melting point (T_m) values are shown as a function of concentration. T_g' and the corresponding solute concentration (C_g') of the unfrozen matrix are observed at the point where T_g and T_m curves coincide (Levine and Slade, 1986). Most reported state diagrams have been presentations of glass transition and melting curves with experimental component T_g, T_g' and C_g' values (Levine and Slade, 1988). For some materials state diagrams with several experimental T_g values at varying water contents have been developed (Blond, 1989; Izzard et al., 1991; Roos and Karel, 1991d).

The glass transition temperature of food components governs physical properties like diffusivity which may also affect rates of deteriorative reactions (Vrentas and Duda, 1978; Simatos and Karel, 1988). Therefore the physical state of an amorphous matrix in frozen and dehydrated foods may greatly influence their stability, and affect both physical and chemical changes during food processing and storage (Levine and Slade, 1988; Roos and Karel, 1991a,b). In polymers molecular weight, composition and plasticizers are the main factors affecting T_g (Fox and Flory, 1950; Gordon and Taylor, 1952; Tant and Wilkes, 1981). They are also important for characterization of amorphous food components (Levine and Slade, 1988; Roos and Karel, 1991b).

Several empirical and theoretical equations relating mechanical properties of polymers, composition and molecular weight to T_g have been reported (e.g. Fox and Flory, 1950; Gordon and Taylor, 1952; Williams et al., 1955; Couchman, 1978; Tant and Wilkes, 1982; Lin et al., 1989). Prediction of T_g of amorphous food components as a function of composition and molecular weight have been reported by Orford et al. (1989, 1990) and Roos and Karel (1991b). Such prediction capability would be useful for food process and product development, and for studies of the effects of physical state on deteriorative changes as a function of temperature, water content and time. In spite of considerable recent work on glass transitions in amorphous food components (e.g. Levine and Slade, 1986; Roos, 1987; Orford et al, 1989; Roos and Karel, 1991a; Slade and Levine, 1991) data and correlations needed for prediction of the physical state are unavailable or controversial. We have previously shown that annealing treatment at $T_g' < T < T_m'$ was necessary to obtain the maximally freeze-concentrated state in sucrose solutions (Roos and Karel, 1991b,d). In our current study we applied similar rigorous procedures with the objective to determine the effect of time-dependent freeze-concentration and molecular weight on the physical state of amorphous food components. Determination of thermal behavior at varying water contents allowed us to predict T_g of polymers for estimating changes in physical state as a function of temperature, water content and molecular weight. We also established state dia-

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grams based on the quantitative experimental and predicted data.

MATERIALS & METHODS

Preparation of samples

Maltose (Sigma, Hydrate Grade I) and maltodextrins (produced from corn starch) of various dextrose equivalents (DE) (Maltrin M040, DE5; M100, DE10; M200, DE 20; M365, DE 35: Grain Processing Corp.) were dissolved in HPLC water (Fisher) as 20% solutions. These were frozen, freeze-dried, dehydrated over P_2O_5, and rehumidified to varying water contents over saturated salt solutions (Roos, 1987; Roos and Karel, 1991b). Samples for studies of T_g' and concentration at maximum freeze-concentration (C_g' [% solute] or W_g' [gH_2O/g solute]) were made by weighing the solids and water to obtain solutions with 20, 30, 40, 70, 75, and 80% of maltose and 20, 30, 40, and 80% solids of maltodextrins. When heating was necessary for solubilization, the solutions were reweighed after a clear solution was obtained, and the amount of evaporated water was added back (Roos and Karel, 1991d). The initial water content of maltodextrins was determined from samples (2g) which had been freeze-dried and dried over P_2O_5. Since maltose and maltodextrins contained 2–5% water, corrected concentrations were used for solutions. Samples of waxy corn starch (Amioca, 98% amylopectin; National Starch and Chemical Corp.) were made by weighing water and starch in DSC pans. The pans were hermetically sealed and the starch was gelatinized in the DSC cell (5 min at 85°C).

Determination of T_g

The glass transition temperature (T_g) and change of specific heat at the T_g region (Δc_p) were determined using differential scanning calorimetry (DSC, Mettler DSC30S) calibrated as reported by Roos and Karel (1991b,d). A scanning rate of 5°C/min was used to analyze samples (10–25 mg in hermetically sealed 40μL aluminum pans; Mettler) over the T_g region as reported by Roos and Karel (1991b). Solutions with 20, 30, and 40% solids were cooled at the maximum (about 30°C/min) rate to −80°C, heated (10°C/min) to an isothermal holding (annealing) temperature, cooled (10°C/min) to −80°C, and scanned from −80°C to 20°C at 5°C/min. Samples were annealed above T_g' but below T_m' to create maximum freeze-concentration for determination of T_g', T_m' and latent heat of melting (ΔH_m) as shown in Fig. 1 and 2, and reported by Roos and Karel (1991d). All solutions were studied for T_g and T_m in nonannealed state, and for the effect of annealing before setting the temperature cycles. The isothermal holding temperature was then set slightly below the concentration-invariant T_m' (15 min at −13°C M040, −15°C M100, −20°C M200, −30°C M365, and −33°C maltose). Gelatinized samples of starch (about 20, 30, and 40% solids) were cooled to −30°C (uncontrolled rate), heated (10°C/min) to −5°C, annealed 15 min at −5°C, cooled (10°C/min) to −80°C, and scanned from −80°C to 20°C at 5°C/min. Solutions with 70, 75, and 80% solids were cooled to −100°C and scanned at 5°C/min to 0°C, and analyzed for T_g onset (T_g^o), midpoint (T_g^m), endpoint (T_g^e), and Δc_p (Fig. 2). The glass transition temperature was defined as the T_g onset value. When T_g' values were determined the onset temperature of glass transition was used as T_g' because melting occurred before conclusion of the second order transition (Fig. 2). All determinations were made in triplicate, and average values were used as transition temperatures.

Prediction of T_g

Gordon and Taylor equation (Eq. 1) relates T_g of miscible polymer blends to the weight fractions and T_g values of component compounds (Gordon and Taylor, 1952). We used Eq. (1) to relate the weight fractions of solute and water (w_1 weight fraction of solute; w_2 ($w_2 = 1 - w_1$) weight fraction of water) and their T_g (T_{g1} for solute (K); T_{g2} for water (138K, Johari et al., 1987)) to the T_g values observed at varying water contents. Couchman (1978) and Couchman and Karasz (1978) showed that the k value in Eq. (1) was equivalent to the ratio of the change of component compound specific heats ($k = \Delta c_{p2}/\Delta c_{p1}$) at their T_g. However, the T_g values predicted with k values calculated using Δc_p values (Table 1) and those of water varying from 0.11 J/g°C to 1.94 J/g°C reported by Hallbrucker et al. (1989) and Sugisaki et al. (1968), respectively, correlated poorly with our experimental T_g values. Therefore experimental T_g values were used to calculate empirical k values. The T_g values developed using empirical values correlated with experimental data, and were used to establish the state diagrams.

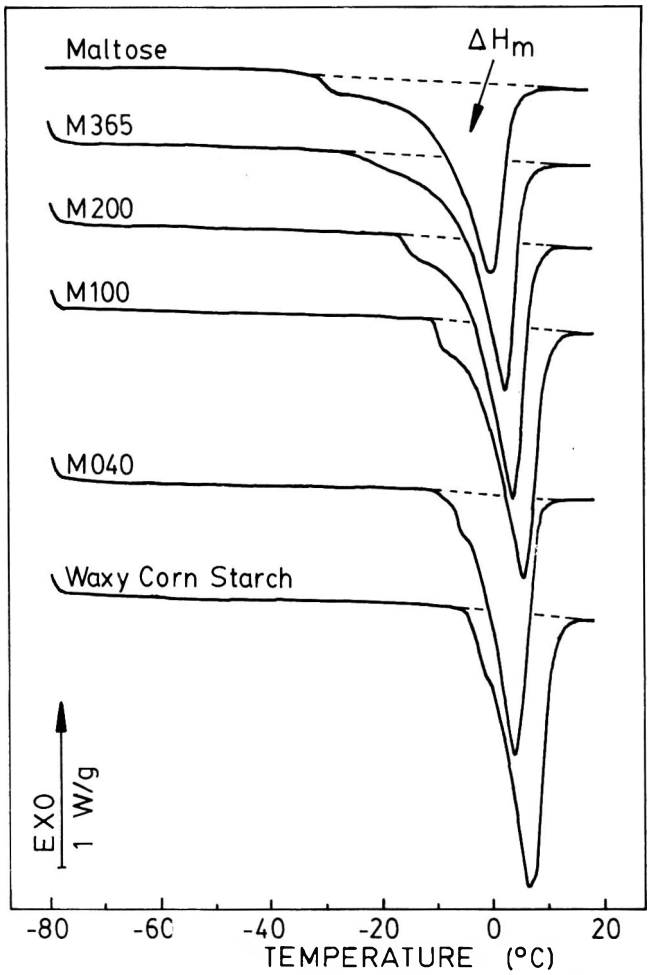

Fig. 1—Thermal behavior of 40% solutions of maltose, maltodextrins and starch annealed 15 min between T_g' and T_m' (Fig. 2) cooled to −8C°C and scanned at 5°C/min to 20°C. Glass transition preceded onset of ice melting, observed as a step change followed by the main melting endotherm. Integration of melting endotherms is shown by dashed lines to determine latent heat of ice melting (ΔH_m).

$$T_g = \frac{w_1 T_{g1} + k w_2 T_{g2}}{w_1 + k w_2} \quad (1)$$

Calculation of effective molecular weight

Maltodextrins are starch hydrolysis products with a wide molecular weight distribution (those we used contained 0.2–6.7% monosaccharides, 0.3–27.8% disaccharides, 0.6–15.4% trisaccharides, 0.6–9.2% tetrasaccharides, and 40.9–98.3% higher than tetrasaccharides depending on DE) and their true T_g values may be lower than those based on average molecular weights. Roos and Karel (1991b) reported that T_g values of M200 were equivalent to those of maltotriose (M = 504 g/mol) and M040 to those of maltohexose (M = 991 g/mol). Thus T_g values of maltose, M200 and M040 were analyzed against inverse values of their effective molecular weights (M_e) which according to Fox and Flory (1950) predicts the effect of molecular weight of homopolymers on T_g (Eq. 2). The calculated M_e values (Table 1) were used to relate T_g' and T_m' values to molecular weights.

$$T_g = T_g(\infty) - \frac{K_g}{M_e} \quad (2)$$

where $T_g(\infty)$ is glass transition of limiting molecular weight (243°C was obtained for maltodextrins) and K_g is a constant (52800 (g/mol) × (°C) was obtained for maltodextrins). $T_g(\infty)$ was also used as T_g for starch.

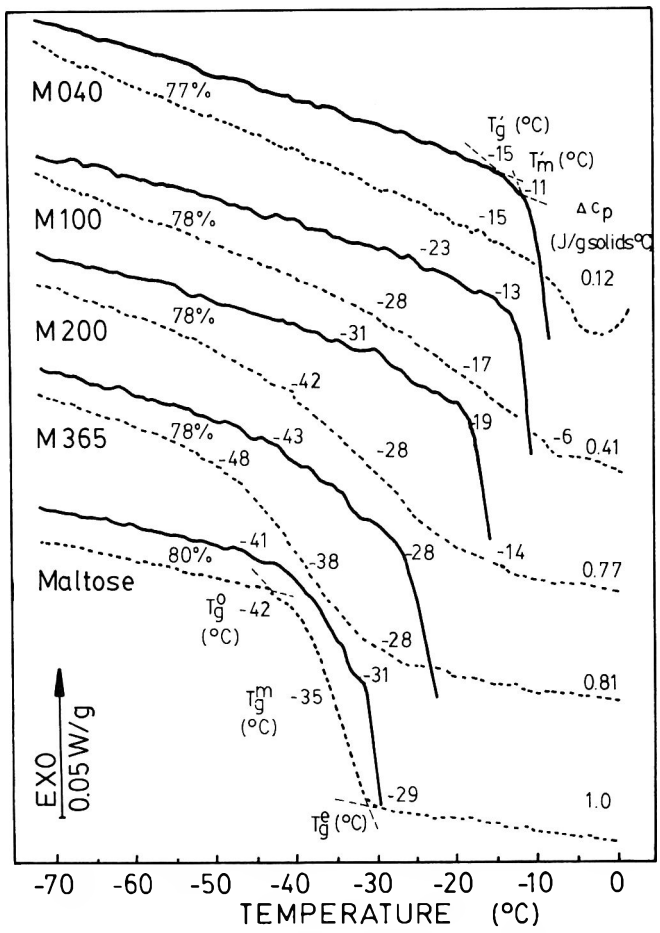

Fig. 2—Expanded thermograms for maltose and maltodextrins showing T_g' and T_m' of annealed (15 min at $T_g' < T < T_m'$) 40% (w/w) solutions obtained at 5°C/min (solid lines). The dashed curves show thermograms obtained at 5°C/min for samples with solute concentrations (77–80% w/w) near the maximum freeze-concentration. T_g^o onset, T_g^m midpoint, and T_g^e endpoint of glass transition region. Δc_p values are changes in specific heat for solutions with 77 to 80% solute.

Determination of unfrozen water

The amount of unfrozen water decreases with increasing freeze-concentration, and annealing above T_g' but below T_m' should lead to maximally freeze-concentrated state of the solutes (Roos and Karel, 1991c,d). Therefore samples were annealed at that temperature range before the amount of unfrozen water was determined. The unfrozen water content was then determined from the linear relationship between latent heat of melting (ΔH_m, [J/g solute] obtained as shown in Fig. 1) and water content [g/g solute], which was extrapolated to $\Delta H_m = 0$ J/g as reported by Simatos et al. (1975) and Roos and Karel (1991d). The unfrozen water contents at maximum freeze-concentration were also calculated from experimental T_g' values using Eq. (1), and the w_1 values were converted to C_g' and W_g' values.

RESULTS

Glass transition temperature

The T_g values for maltose and maltodextrins dried over P_2O_5 are given in Table 1 with respective Δc_p values. The decrease of T_g with increasing water content was substantial as shown in the state diagrams (Fig. 3 and 4). Ice formation was not likely in samples containing more than 80% solids (Fig. 2). We were not able to determine T_g values for completely unfrozen matrices with 20, 30 or 40% solids because ice formation occurred even in samples quenched in liquid nitrogen.

T_g of freeze-concentrated matrices

Annealing of solutions with 20, 30 and 40% solute led to constant, initial-concentration-independent T_g' and T_m' values. The constancy of these values was considered an indication of maximum ice formation. No devitrification (crystallization of unfrozen water) exotherms were observed in the rewarming thermograms of the annealed samples (Fig. 1 and 2). Thus the T_g' and T_m' values were considered as T_g' and T_m', respectively. The T_g' values were near the T_g values of 78–80% solutions (Fig. 2) which remained unfrozen.

Effect of molecular weight on T_g

The T_g of maltodextrins increased with increasing molecular weight in agreement with the Fox and Flory -equation (Eq. 2) when effective molecular weights based on equivalent T_g values of maltodextrins, maltotriose and maltohexaose were used. T_g of high molecular weight maltodextrins decreased more steeply with increasing water content than that of maltose or maltodextrins with lower molecular weights (Fig. 3 and 4). The T_g' values of maltodextrins increased with increasing effective molecular weight (Fig. 1). Both T_g' and T_m' increased with increasing molecular weight (Fig. 2) and showed linearity with inverse effective molecular weight (Fig. 5) as reported by Levine and Slade (1986). This relationship indicated that T_g' and T_m' for high molecular weight maltodextrins should coincide at -1.0°C corresponding to an effective molecular weight of 5265 g/mol.

Prediction of glass transition temperature

Prediction of T_g values of polymer mixtures with Eq. (1) required component T_g values, weight fractions and k values. The k values obtained using component Δc_p values were much lower than the empirical k values (Table 1) which would have led to highly erroneous T_g values if used for T_g prediction. However, the Δc_p values of maltodextrins decreased with increasing molecular weight (Table 1) which should lead to increasing k values with increasing molecular weights. The empirical k values resulted in a prediction for specific heat change of water of 3 ± 0.5 J/g°C at T_g.

Table 1—Effect of molecular weight on glass transition (T_g) and change of specific heat at T_g (Δc_p, ΔC_p) for anhydrous maltose and maltodextrins

Compound	M[a] (g/mol)	M_e[b] (g/mol)	T_g (°C)	Δc_p (J/g)	ΔC_p (J/mol)	k[d]	k[e]
Water	18.0	18.0	−135[c]	[c]			
Maltose	343.2	343.2	87	0.61	209	6	3.2 (0.18)
M365	500	370	100	0.58	215	6	3.3 (0.19)
M200	900	504	141	0.45	227	6.5	4.3 (0.24)
M100	1800	640	160	0.40	256	7	4.8 (0.28)
M040	3600	991	188	0.30	297	7.7	6.5 (0.37)

[a] Molecular weight or that indicated by manufacturer.
[b] Effective molecular weight.
[c] T_g = −135°C (Johari et al., 1987); Δc_{p2} = 1.94 J/g°C (Sugisaki et al., 1968) and 0.11 J/g°C (Hallbrucker et al., 1989).
[d] Empirical k value for Eq. (1).
[e] $k = \Delta c_{p2}/\Delta c_{p1}$; Values in parenthesis are those with Δc_{p2} = 0.11 J/g°C.

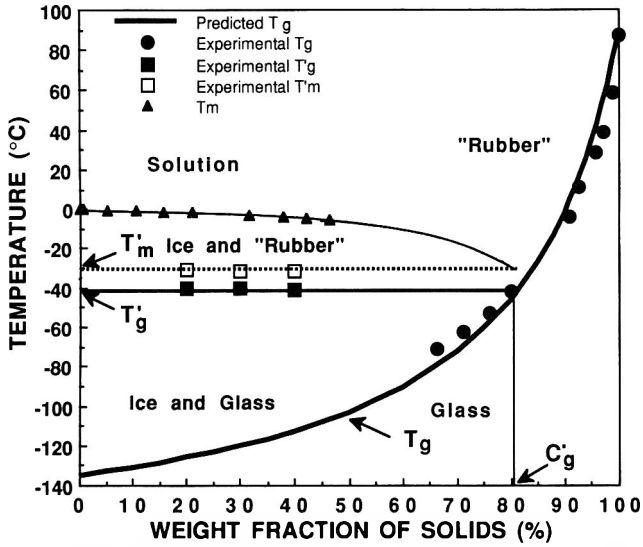

Fig. 3—State diagram for maltose. T_g curve predicted by the Gordon and Taylor equation (Gordon and Taylor, 1952). Below T_g maltose solutions are in the glassy state. Maximally freeze-concentrated solutions show constant T'_g (onset of glass transition) and T'_m (onset of ice melting) values. The T'_m of maltose was at the endpoint region of T'_g. Maximum ice formation occurred at temperatures $T'_g < T < T'_m$. T_m values are from Weast (1974).

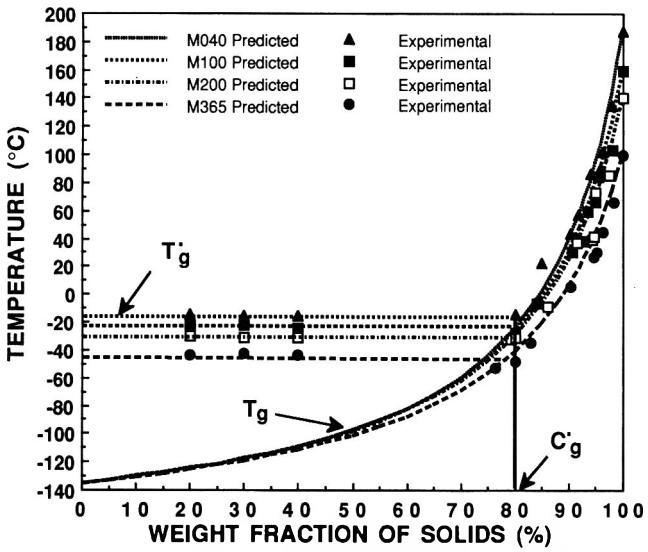

Fig. 4—State diagram for maltodextrins. T_g decreased with decreasing molecular weight and increasing water content. Above 50% the T_g values were predicted to be almost independent of molecular weight. The T'_g value increased with increasing molecular weight but was predicted to become constant for high molecular weight maltodextrins.

Unfrozen water at maximum freeze-concentration

The unfrozen water contents were obtained by plotting ΔH_m values [J/g solute] against water content [gH_2O/solute] and extrapolating to $\Delta H_m = 0$ J/g solute (Table 2). The slope increased with increasing T'_g and T'_m values, which indicated decreasing latent heat of melting with decreasing melting temperature. Note that integration of melting peaks of annealed samples was done from $T < T'_m$ as shown in Fig. 1. Annealing of starch solutions probably did not result in maximum freeze-concentration.

The use of T'_g values and Eq. (1) was considered as the most precise method for prediction of unfrozen water contents of

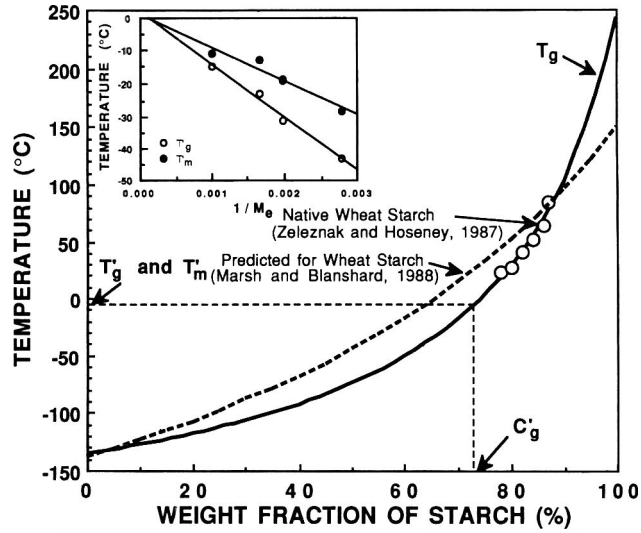

Fig. 5—State diagram for starch based on T_g at 243°C and experimental data of Zeleznak and Hoseney (1987). The T'_g and T'_m values of high molecular weight solutes were predicted to have the same temperature value as shown in the inset figure ($T'_g \leq T'_m$). Solute concentration of maximally freeze-concentrated state was predicted lower than that of maltodextrins. The predicted values of Marsh and Blanshard (1988) are shown for comparison.

the maximally freeze-concentrated matrices (Table 2) since T_g values were extremely sensitive to changes in water content (Fig. 2 to 5). The T'_g values increased with increasing molecular weight but the C'_g was almost constant (about 80% for maltose and maltodextrins, Fig. 3 and 4). The unfrozen water content calculated for starch was higher (about 27%, Fig. 5) in agreement with the measured 27% reported by Slade and Levine (1987).

State diagrams

State diagrams were developed for maltose (Fig. 3) and for maltodextrins (Fig. 4). T'_g and T'_m were clearly separated with maltose (Fig. 1) and ice melting had onset at the end point region of glass transition. Maltodextrins showed decreasing temperature differences between T'_g and T'_m with increasing molecular weight (Fig. 5), and for starch $T'_g = T'_m$ was assumed. The state diagrams were constructed with both predicted and experimental T_g values for the entire concentration range (Fig. 3 to 5). The projected T_g of anhydrous starch was 243°C. T_g values reported for native wheat starch by Zeleznak and Hoseney (1987) were used to determine k for starch (Fig. 5), and k = 5.2 was used in calculations.

DISCUSSION

PHYSICAL CHANGES e.g. crystallization of amorphous sugars, stickiness and collapse of dehydrated materials are directly related to T_g (Levine and Slade, 1986; Roos and Karel, 1990; Roos and Karel, 1991a). Roos (1987) pointed out that the T_g of freeze-dried strawberries decreased linearly with increasing vapor pressure of water used for rehumidification of samples at 25°C (water activity, a_w). This was later reported also for amorphous sugars, sugar mixtures and maltodextrins although through the total a_w range the relationship was found to be sigmoid (Roos and Karel, 1991a,b). The linearity between a_w and T_g in the a_w range 0.1–0.8 allows prediction of T_g at the a_w range typical of dehydrated and intermediate moisture foods. It is useful when materials are evaluated for drying characteristics or stability at varying storage conditions (Roos, 1987; Slade and Levine, 1991).

Food composition is often modified to improve material

Table 2—Glass transition temperature (T_g'), ice melting temperature, (T_m'), and unfrozen water (G_g', W_g') of maximally freeze-concentrated solutions

Compound	T_g' (°C)	T_m' (°C)	ΔH_{mw}^a (J/gH_2O)	C_g' (% solute) I[b]	C_g' (% solute) II[c]	W_g' (gH_2O/g solute) I[b]	W_g' (gH_2O/g solute) II[c]
Maltose	−41	−31	295	75.2	82.5	0.33	0.23
M365	−43	−28	297	78.1	79.4	0.28	0.26
M200	−31	−19	308	78.7	79.7	0.27	0.25
M100	−23	−13	314	78.1	81.1	0.28	0.23
M040	−15	−11	323	70.9	82.0	0.41	0.22
Starch		−6	313	79.4	72.9	0.26	0.37

[a] Estimated latent heat of ice melting.
[b] Extrapolated concentration from ΔH_m values.
[c] Calculated concentration using Eq. (1): $T_g' = (w_1 \times 100)\%$ at T_g'

characteristics in processing and storage. In our study we have reported results for T_g values of food components of varying molecular weights and methods for prediction of T_g over a full concentration range. Eq. (1) is one of the basic relationships used to predict T_g values for polymer mixtures. Several modifications of the equation have been reported (Couchman, 1978; Couchman and Karasz, 1978; Lin et al., 1989) and used for food related materials (To and Flink, 1978; Marsh and Blanshard, 1988; Orford, 1989; Roos and Karel, 1991b). Ellis (1988) reported a good correlation between experimental and predicted T_g values for T_g of amorphous polyamides as a function of water content. They used component Δc_p values in Eq. (1), and the value reported by Sugisaki et al. (1968) (1.94 J/g°C) was used for water. Orford et al. (1990) also used component Δc_p values to correlate predicted and experimental T_g values of amorphous carbohydrates. A linear relationship between mole fractions of water and T_g was observed but the correlation with experimental and predicted T_g values was poor. Also a wide range of Δc_p values have been reported for amorphous water which makes the use of Δc_p values controversial. Our results showed that empirical k values can be successfully used for prediction of T_g as a function of water content.

In some cases T_g values of high molecular weight materials are difficult to analyze because of broadening of the transition, relatively small Δc_p, and decomposition (Zeleznak and Hoseney, 1987; Roos and Karel, 1991b). Broadening of T_g is typical of polymer mixtures (Ellis, 1988). We noticed broad T_g regions for dehydrated maltodextrins (Roos and Karel, 1991b) and for freeze-concentrated maltodextrin solutions (Fig. 2). Thus prediction of T_g values is important in analysis of compositional and molecular factors on T_g. The effect of molecular weight on T_g can be used to predict T_g values of homopolymers. Our predicted T_g for anhydrous starch (243°C) was 100°C above T_g used by Marsh and Blanshard (1988) (Fig. 5) but comparable with 250°C proposed by Biliaderis et al. (1986) and 227°C predicted for amylose and amylopectin by Orford et al. (1989). Gelatinization of starch occurs typically at a temperature range of 50–90°C and the minimum water needed for gelatinization is about 30% (Biliaderis et al., 1986; Slade and Levine, 1987). Thus the T_g of gelatinized starch solutions (gelatinization occurs in solubilization) are below −20°C but the effective T_g controlling gelatinization before solubilization may be considerably higher (Biliaderis et al., 1986; Slade and Levine, 1987).

T_g' is the effective glass transition for maximally freeze-concentrated matrices at temperatures below T_m'. Partially freeze-concentrated solutions can persist only at temperatures below T_g' or above T_m' (Roos and Karel, 1991d). Freezing to a maximally freeze-concentrated state was considered time-dependent and to require annealing at a temperature favoring maximum ice formation (Roos and Karel, 1991c,d). We expected that maximum amount of ice could be obtained only by annealing at a temperature above T_g' but slightly below T_m'. These temperatures were considered as limits for maximum freeze-concentration because crystallization of water below T_g' probably proceeds only to a concentration having T_g at that temperature. Above T_m' melting dilutes the unfrozen matrix. Maltose and low molecular weight maltodextrin solutions had relatively low T_g' and T_m' values. As shown by the T_m curve (Fig. 3) the equilibrium solute concentration of partially freeze-concentrated maltose solutions at −20°C was about 70% with effective T_g at about −70°C. According to Williams-Landel-Ferry (WLF) relationship (Williams et al., 1955) at 50°C above T_g viscosity is decreased from 10^{11} Pa s to 10^2 Pa s (Roos and Karel, 1991d). This may lead to Arrhenius type temperature dependence of deteriorative changes in partially freeze-concentrated low molecular weight carbohydrate solutions above T_m'. T_g' and T_m' values of high molecular weight maltodextrins were confirmed to be relatively high, and in this case storage at normal freezer temperatures and below T_g' may improve shelf life as proposed by Levine and Slade (1986, 1988).

Low molecular weight compounds in carbohydrate mixtures may significantly decrease the effective T_g of high molecular weight compounds (Orford, 1990; Roos and Karel, 1991b). The T_g' increased with increasing molecular weight but based on Eq. (1) fairly high amounts of high molecular weight compounds must be added to materials with low T_g' to increase the effective T_g' to normal frozen food storage temperatures. However, Muhr and Blanshard (1986) showed that crystallization of water in sucrose solutions was delayed by addition of polysaccharides. In frozen foods the effective T_g' may be relatively low but the viscosity can be increased by addition of high molecular weight compounds which may delay crystallization or other diffusion limited changes. High molecular weight solutes in our study also seemed to increase T_m' values, and T_g' values were probably related to the specific materials freezing point depression of water.

The temperature difference between T_g' and T_m' decreased with increasing molecular weight, and T_g' and T_m' for high molecular weight compounds were predicted to have the same temperature value. Although starch solutions were annealed at −5°C melting was observed at about −6°C. This may have been caused by nonequilibrium conditions during a simultaneous first order (ice melting) and second order (glass transition) phase transition, which caused annealing at $T_g' = T_m'$ to become ineffective. T_g' of starch at −5°C could not be proven with a solution having an approximate concentration of the maximally freeze-concentrated state. However, our T_g' value agreed with T_g' (−5°C) reported for starch and those of low DE maltodextrins (Levine and Slade, 1986; Slade and Levine, 1987). This was also consistent with our prediction showing that above a certain molecular weight the T_g' and T_m' values became independent of the molecular weight of solute as was also pointed out by Levine and Slade (1986). Generally our T_g' midpoint values were lower than those reported by Levine and Slade (1986, 1988). They were found to be slightly above our T_m' (e.g. for maltose T_g' was −41°C and T_m' was −31°C) while Levine and Slade (1988) reported T_g' at −29.5°C.

As indicated solute concentration at maximum freeze-concentration was determined by extrapolation of ΔH_m values to $\Delta H_m = 0$ J/g, and from state diagrams. However, when unfrozen water was calculated from single ΔH_m values (334 J/g for water) as proposed by Levine and Slade (1986, 1988) it decreased with increasing concentration as previously reported

by Simatos et al. (1975), Roos (1987), Blond (1989), and Roos and Karel (1991d). Unfrozen water estimated by extrapolation of ΔH_m values was in most cases higher than that determined from state diagrams (Table 2). Solute concentration estimated from state diagrams was about 80% independently of molecular weight. the unfrozen water contents by extrapolation agreed reasonably well with those from Eq. (1). However, the ΔH_m values depend on annealing treatment.

Second order transitions in food components are most important to processes in which water is removed by evaporation or freezing, and to physical properties of materials produced with such methods (e.g. Levine and Slade, 1986; Roos, 1987; Roos and Karel, 1991a,b; Slade and Levine, 1991). In food dehydration the rapid change of T_g with minor changes in moisture content may cause significant changes to product quality (Roos and Karel, 1990; Slade and Levine, 1991). In freezing, an intermediate amount of moisture remains in the unfrozen matrix which contains most of the compounds capable of causing deterioration during storage. In our study state diagrams based on both experimental and predicted transition temperatures were established. State diagrams showing the T_g values over a wide concentration range have been reported on few compounds because of the uncertainty of T_g values of unfrozen matrices with concentrations lower than C_g'. Knowledge of the T_g values of partially freeze-concentrated states is extremely important for studies of frozen food stability. However, values of T_g at concentrations well below C_g' are difficult to obtain because of rapid freeze-concentration. Predicted T_g values at high water contents seemed to become almost equal and independent of molecular weight (Fig. 4). Thus the temperature of frozen food storage may significantly affect stability above T_g' (Levine and Slade, 1986) and T_m'. State diagrams showing T_g of food components over the entire concentration range are needed for successful control of second order transition in food engineering operations.

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Water Retention Capacity and Viscosity Effect of Powdered Cellulose

JIT F. ANG

ABSTRACT

Powdered cellulose is a natural beta-1,4-glucan polymer. It is 99% total dietary fiber (dwb) and has many uses as a low-calorie food ingredient. Depending on its fiber length, cellulose can retain 3.5 to 10 times its weight in water. Temperature and pH exerted small effects on water rentention capacity. A powdered cellulose with a 110-micron fiber length significantly increased viscosity. Its ability to increase viscosity was further magnified in solutions containing low amounts of stabilizers. This fiber may act synergistically with other stabilizers to increase viscosity.

Key Words: cellulose, viscosity, water-retention, thickening-agent, low-calorie

INTRODUCTION

DIETARY FIBER and low-calorie foods have recently received considerable attention from nutritionists, medical authorities, and food processors, as well as the general public. Coupled to this increasing awareness and interest is an expansion in the market for related food ingredients. More recently, ingredients such as wheat bran, oat bran, potent sweeteners, fat substitutes, and other similar products have been studied for properties and potential applications in foods.

Powdered cellulose, a natural beta-1,4-glucan polymer, has been used as a food ingredient for many years (Ang et al., 1988). Since it is an insoluble fiber with an average total dietary fiber content of not less than 99% (dwb), powdered cellulose is also considered to be noncaloric (FDA, 1987). This principal structural component of trees and other plants is the most abundant source of complex carbohydrate in the world. Although some studies have been conducted on the use of cellulose as a food, most of them were focussed on its physiological effects (Reiser, 1984) rather than its properties as a food ingredient. Due to its chemical composition, powdered cellulose is widely used as a non-caloric bulking agent in reduced-calorie foods. Since water also plays a significant role in such products, our objective was to investigate some physical properties of powdered cellulose: water retention capacity and effect on viscosity.

MATERIALS & METHODS

Materials

Samples of powdered cellulose (Solka-Floc^R) with varying average fiber lengths were obtained from Fiber Sales and Development, Berlin, NH. Fiber lengths reported represent the most typical values (from data collected over 2 yr). Variations in fiber length are normally limited to ±20%. Guar gum (Uniguar 80) was obtained from Celanese, Clifton, NJ; carboxymethylcellulose (CMC 9M31XF) from Hercules, Wilmington, DE; and xanthan gum (E415) from Jungbunzlauer, Germany. The pH buffers were purchased from Fisher Scientific (Pittsburgh, PA). All other chemicals were of analytical or food grade.

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Water retention capacity

Using a glass rod, 2g samples of powdered cellulose were mixed with 30 mL of distilled deionized water contained in 50 mL centrifuge tubes. The slurry was allowed to stand for 10 min, then centrifuged at 2000 × *g* for 15 min in an International Centrifuge (Model SBV). After centrifugation, the supernatant solution was drained and the wet cellulose precipitate was weighed. Since powdered cellulose is not soluble (FCC, 1981), results were expressed as g water retained/g sample.

To study the effect of pH on water rentention capacity (WRC), similar experiments were carried out. However, slurries were made with commercial buffers (pH 2 to 10) standardized vs reference materials from the National Bureau of Standards. The pH of each slurry was checked with a pH meter prior to centrifugation, and adjustments, if necessary, were made using 0.1N HCl and/or 0.1N NaOH.

To study the effect of temperature on water retention, distilled deionized water at two different temperatures (4°C and 70°C) was used. In these experiments, a Beckman L5-65B Ultracentrifuge was used and the temperature of the centrifuge chamber was set to maintain the temperatures studied.

Corn oil retention capacity

The procedure for this test was similar to those described for water retention capacity. Using a glass rod, 2 g samples of powdered cellulose were mixed with 30 mL liquid corn oil. This slurry was treated as outlined above. Results were expressed as g corn oil retained/g sample.

Viscosity measurements

Solutions containing 1, 2, and 3% (w/v) cellulose were prepared by blending appropriate amounts of cellulose in distilled deionized water. An even dispersion was achieved with the aid of a Brookfield counter-rotating mixer. Viscosities of the slurries were then measured with a Brookfield digital viscometer (Model DV-1) fitted with spindle No. 1 at 60 rpm. All experiments were carried out at room temperature and results were expressed in cps.

To study the effect of powdered cellulose on viscosities of solutions containing low amounts of stabilizers, cellulose at 1, 2, and 3% (w/v) was added to solutions containing 0.1%, 0.2%, and 0.3% (w/v) guar gum, carboxymethylcellulose (CMC), or xanthan gum. Apparent viscosities were measured at 6 to 60 rpm with a No. 1 spindle.

Statistical analysis

Analysis of variance on the data was made using the Multiple Comparison Test. Significant differences ($p < 0.05$) among sample means were determined by the Tukey's Test. A minimum of 6 duplicate runs were conducted on separate occasions.

RESULTS & DISCUSSION

Water and corn oil retention capacities

The data depicting the effect of fiber length on the WRC of powdered cellulose are presented in Fig. 1. Average fiber lengths were determined by staining cellulose fibers with "C" Stain (Graff, 1940) and then measured under a light microscope (Kluchnik, 1981). WRC was observed to increase with increasing fiber length. Eight fiber lengths were used (11, 17, 22, 35, 50, 60, 110, and 290 microns). Depending on its fiber length, powdered cellulose retained about 4.0 to 10 times its

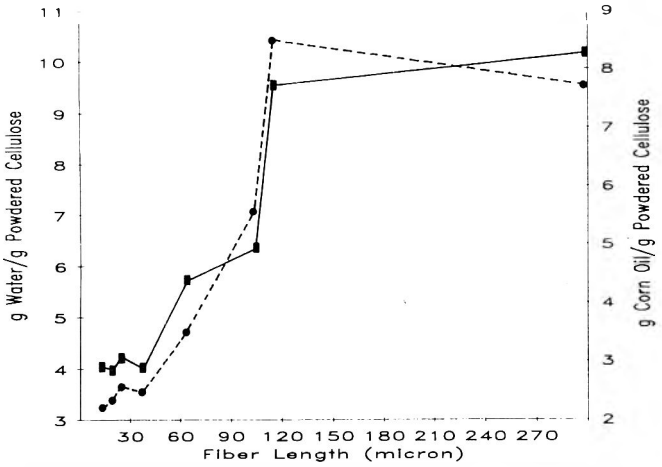

Fig. 1—Effect of fiber length on the water and corn oil retention capacities of powdered cellulose. ■———■ water; ●--------● corn oil.

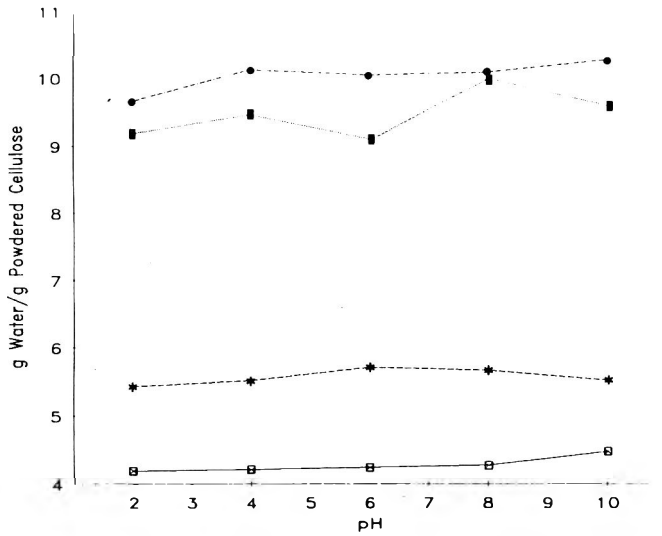

*Fig. 2—Effect of pH on the water retention properties of powdered cellulose. ☒———☒ 22 micron; *———* 60 micron; ■ . . . . . ■ 110 micron; ●-------● 290 micron.*

Table 1—Viscosities of powdered cellulose suspended in water

Fiber length of cellulose (μ)	Concentration of cellulose (% w/v)			
	0	1	2	3
35	1.0[a]	0.8[a]	1.0[a]	0.7[a]
60	1.0[a]	1.5[a]	1.3[a]	1.5[a]
110	1.0[a]	5.0[b]	10.4[c]	27.3[d]

[a-d] Means having the same superscript do not differ significantly ($p < 0.05$).

weight in water. Two plateaus were observed in Fig. 1. The WRC of cellulose with fiber lengths greater than 110 microns did not vary as much as those with fiber lengths between 35 and 100 microns. Below 35 microns, WRC was also less dependent on fiber length.

A similar pattern was obtained when water was substituted with liquid corn oil (Fig. 1). However, retention capacities for corn oil were generally lower than those for water. Powdered cellulose with fiber lengths between 22 and 290 microns retained only about 2.5 to 8.5 times its weight in corn oil.

Cadden (1987) reported that decreasing the particle size of certain fibrous ingredients, such as wheat bran, decreased their WRC. However, other fibers including oat bran and microcrystalline cellulose also reported demonstrated higher WRC when their particle sizes were reduced. Figure 1 indicates that

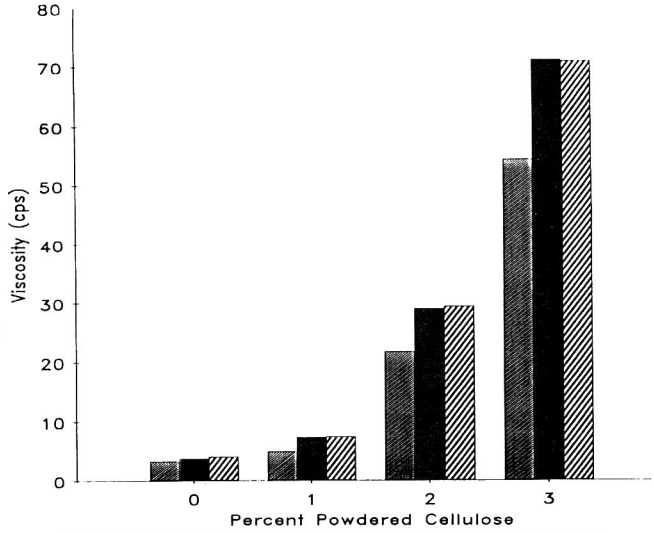

Fig. 3—Effect of 110-micron powdered cellulose on the viscosity of guar gum solutions. ▨ 0.1% Guar; ■ 0.2% Guar; ▨ 0.3% Guar.

the WRC of powdered cellulose was more similar to those of wheat bran. The data also demonstrate the importance of physical structure on WRC. Cellulose is a porous fiber and if this fiber matrix is altered, its water imbibing properties would be affected. Since powdered cellulose is a mixture of amorphous (30%) and crystalline cellulose (70%) (Penner, 1989), a substantial portion of the fiber exists in the porous matrix form. On the other hand, microcrystalline cellulose is comprised of mostly crystalline cellulose (more than 80%). Therefore the role of this porous matrix in WRC for microcrystalline cellulose is not as important.

Effects of pH and temperature on WRC

The effect of pH on WRC is shown in Fig. 2. Cellulose with various fiber lengths was analyzed, but for the sake of clarity, only 4 fiber lengths are reported, i.e. 22, 60, 110, 290 microns. Data obtained suggested that pH had a small effect on WRC. Powdered cellulose was observed to retain more water at higher pH. In addition, the effect of pH on WRC was greater in cellulose with longer fiber lengths. Parrott and Thrall (1978) reported that the WRC of a 120-micron powdered cellulose was unaffected by pH. In addition, they reported that cellulose with a fiber length of about 40 microns exhibited maximum and minimum WRC at specific pH values. Results in Fig. 2 did not confirm the findings of Parrott and Thrall (1978). We also observed that within the range 4 to 70°C, temperature had no significant effect on WRC (data not shown).

Viscosity

With the exception of cellulose with a 110-micron fiber length, powdered cellulose did not appear to have thickening properties when suspended in water (Table 1). The viscosities of suspensions containing a 110-micron cellulose fiber increased significantly with increasing cellulose concentrations. With a 1% suspension, viscosity was magnified 5-fold, while in a 3% suspension viscosity increased about 27 times. However, we observed that, upon setting without agitation, the cellulose fibers settled out of suspension. Therefore, we concluded that over a long period of time, the use of other stabilizers was needed to hold cellulose fibers in suspension.

The effect of this 110-micron cellulose on viscosity was further investigated in solutions containing low amounts of conventional stabilizers. Results depicting the effect of this fiber on solutions containing guar gum (Fig. 3), CMC, (Fig. 4), and xanthan gum (Fig. 5) are shown. In all cases, solution

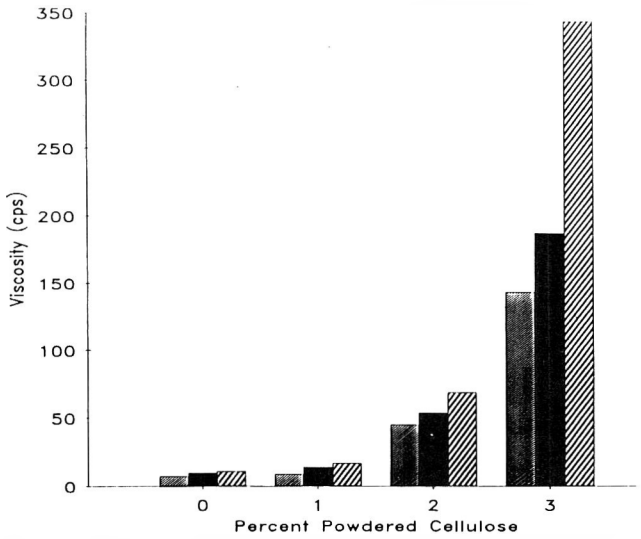

Fig. 4—Effect of 110-micron powdered cellulose on the viscosity of CMC solutions. ▨ 0.1% CMC; ■ 0.2% CMC; ▨ 0.3% CMC.

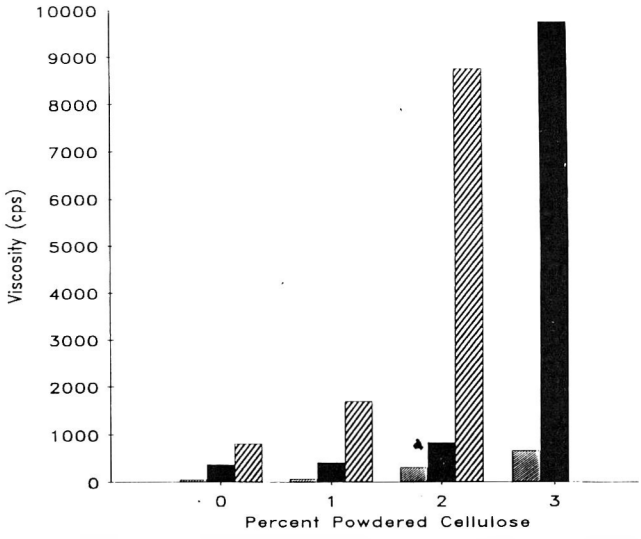

Fig. 5—Effect of 110-micron powdered cellulose on the viscosity of xanthan gum solutions. ▨ 0.1% Xanthan; ■ 0.2% Xanthan; ▨ 0.3% Xanthan.

viscosities were significantly increased by addition of this 110-micron cellulose. The viscosity for 3% cellulose in 0.3% xanthan solution was not reported since the resulting viscosity was in excess of the measurable range.

Data suggest that the use of thickening agents in combination with this cellulose fiber may act synergistically, increasing viscosity in a nonlinear manner. These findings are interesting since no previous research relating the effect of powdered cellulose on solution viscosity has been reported.

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Rheological Changes in Oatmeal Suspensions During Heat Treatment

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ABSTRACT

Two amylases from fungal and bacterial source were added to oatmeal suspensions in concentrations varying from 0.0125 to 0.64%. Changes in viscosity, phase angle and elastic modulus during heating were followed together with sedimentation and flow properties. The complete pasting behavior could be followed without sedimentation problems. The hydrocolloidal properties of β-glucans appeared to be important. The bacteria/enzyme reduced peak viscosity much more rapidly than the Fungamyl enzyme at low concentrations. The flow properties of a Fungamyl treated oat meal suspension with the peak viscosity reduced > 90% were pseudoplastic.

Key Words: rheology, oatmeal suspensions, viscosity, sedimentation, enzymes, amylase

INTRODUCTION

THE COMPOSITION and nutritional value of oats indicate that it is a food substance of great importance (Lockhart and Hurt, 1986). The reports on lowering of blood cholesterol level combined with a more stable glucose level are of special interest. Those benefits are reported to be a result of the high fiber content of β-glucans (cf. Mälkki et al., 1990). A cereal with such advantages needs to be further characterized in order to facilitate increased human consumption. The utilization of food oats is dominated by rolled oat flakes and whole oatmeal. Oats are also used as animal feed, which accounts for over 90% of the harvest in e.g. Sweden and the United States.

The purpose of our study was to follow the rheological changes which occur in an oatmeal suspension during heating. Few fundamental rheological measurements on oats are published. The equipment most frequently used, the amylograph, registers the resistance to shear in Brabender Units, BU, which is not a standard viscosity unit (Atwell, 1986). Direct comparisons of viscosity in appropriate terms with other polysaccharide or polymeric liquids could be valuable.

A dry matter content of 15 to 20% whole oat flour was chosen to simulate a level used in industrial processes, such as gruel production. The parameters were chosen to simulate fast heat treatment of an oatmeal suspension such as that which occurs in a scraped surface heat exchange. In earlier studies, lower concentrations of purified starch or preheated starch suspensions were used above gelatinization temperature before rheological measurements began (Evans and Haisman, 1979; Bagley and Christianson, 1982).

Addition of α-amylase enzymes to the oat meal suspensions was also studied. High dry matter content was achieved with an acceptable increase of viscosity in the oat meal suspension during heating. The type and behaviour of the amylase is very important for the resulting breakdown of oat starch into smaller carbohydrates and ensuing rheological changes (Lindahl, 1989). A suitable α-amylase combined with desired rheological changes also may contribute to the natural sweetening of the food.

Oscillatory measurements during heating of an oatmeal suspension were also used in our study in order to determine the viscosity with very small deformations. This has the benefit of being nondestructive to the gel when small amplitudes are used. Such results have been reported for pure starch suspensions from samples other than oats (Eliasson, 1986).

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MATERIALS & METHODS

Oatmeal

Oatmeal composed of Swedish oat varieties for commercial use was supplied by Kungsörnen AB, Järna, Sweden. The oat kernels were treated by allowing them to descend slowly through a tower (Darre/Granotherm, Bühler, Switzerland). At the top of the tower the oats were treated with directly injected steam at 100°C to inactivate lipid oxidizing enzymes. After 1 hr of passage through the tower the kernels were cooled to room temperature. Before the kernels were rolled to flakes, they were treated again with steam to soften them. The flakes were then ground to oatmeal. Chemical analyses provided by the Swedish Cereal Laboratory gave a composition of 12.2% protein (AOAC, 1980), 71.6% starch (Åman et al., 1989), 8.3% fat (OJEC, 1982), 6.5% damaged starch (AACC, 1960), and 1.7% sugars (method developed by the Swedish Cereal Laboratory). In house assays using standard methods resulted in: 2.0% ash (AACC, 1960) and 9.4% moisture (AOAC, 1980).

Enzymes

The α-amylases were purchased from Novo Industry Enzyme Division, Denmark. Amylases of fungi and bacterial origin were used. Fungamyl 800 L is a purified fungal α-amylase produced from a selected strain of *Aspergillus oryzae*. Optimum temperature was 55°C. The residual activity of this enzyme is close to zero after 15 min at 70°C. BAN 120 L is a bacterial α-amylase produced by submerged fermentation of a selected strain of *Bacillus subtilis*. The optimum relative activity temperature is 72°C. Its residual activity is 95% after 15 min at 70°C.

Sample preparation

Suspensions of oatmeal were prepared from 15 to 20% (w/w) with tap water at room temperature (22.5 ± 1°C). The oatmeal/water suspensions (30 mL) were held at 50°C while stirring for 5 min before being poured into the measuring cylinder of the rheometer. All additions of amylases were done into 20% (w/w) oat meal/water mixtures. The bacterial amylase (BAN 120 L) was added from 0.025% to 0.42% of the total sample weight and the fungi amylase (FUNGAMYL 800 L) from 0.025% to 0.64%.

The Fungamyl enzyme was added prior to and the BAN enzyme was added after the oatmeal suspensions were warmed. For comparison, experiments were carried out with the addition of the Fungamyl enzyme after the oatmeal suspensions were warmed. The separate addition of the amylases was due to their different temperature optima, 55°C for the fungal and 72°C for bacterial amylases. Activity experiments were carried out using 0.1% BAN enzyme. The enzyme was heated at 95°C for 10 min and then added to a 20% oatmeal suspension. Viscosity was measured in the rheometer.

Differential scanning calorimetry (DSC)

A Perkin Elmer DSC II calorimeter was used for determination of thermal transition enthalpies. The samples were weighed into DSC aluminium pans and mixed at a ratio of 1:4 (oatmeal to water, w/w). After sealing the pans, they were heated from 22 to 102°C at 10°C/min. An empty pan was used as reference.

Rheological measurements

The rheological properties were analyzed in the Bohlin Rheometer System (Bohlin Reologi AB, Lund, Sweden). A wide-gap concentric-cylinder system, (i.e. with a ratio between the radii of the inner and the outer cylinders less than 0.97) was used; the outer cylinder rotated during measurements (Barnes et al., 1989). The inner cylinder had a conical bottom with an angle of 150°.

Viscosity measurements below pasting temperature. A wide-gap system with ratio between radii of the inner and outer cylinders of 0.51 was used with a sample volume of 30 mL. The viscosity was measured at a fixed shear rate, 1.72 sec^{-1}. The measurements were done on a 20% oatmeal suspension without added enzyme. The temperature was held constant at 22°C. Viscosity was measured continuously for 3½ hr, then the sample was resuspended and the viscosity measured for an additional 15 min. For comparison a similar experiment was carried out at 50°C in which viscosity was measured continuously for more than 1 hr.

Viscosity measurements during pasting. A wide-gap system with a ratio between the radii of the inner and outer cylinders of 0.51 was used with a sample volume of 30 mL. The viscosity was measured at a fixed shear rate, 1.72 sec^{-1}. The rheological equipment was warmed to 60°C before measurement began. During measurements the temperature was increased from 60 to 90°C, held at 90°C for 10 min, and then decreased to 30°C (Wikström Jansson, 1990). The temperature gradient was 6°C/min, the maximum capacity of the equipment. The viscosity was measured continuously.

Oscillatory measurements. The oscillatory measurements were performed using a wide-gap system with a ratio between the radii of the inner and outer cylinders of 0.91 on a 20% oatmeal suspension without added amylase. A sample volume of 15 mL was used. The frequency was 0.5 Hz at a strain of $8 \cdot 10^{-3}$. The viscosity was calculated from the relation

$$\eta = \frac{G''}{\omega}$$

where G″ is the loss modulus and ω is the frequency in rad/sec. The phase angle, δ, was calculated by the equation

$$\tan \delta = \frac{G''}{G'}$$

where G′ is the elastic modulus. The temperature was increased from 52°C to 90°C at 6°C/min and held at 90°C for 10 min.

Flow behavior. A wide-gap system with a ratio between the radii of the inner and outer cylinders of 0.51 was used with a sample volume of 30 mL. The viscosity was measured during an increase of the shear rate from $6.86 \cdot 10^{-2}$ sec^{-1} to $1.72 \cdot 10^{2}$ sec^{-1}, while the temperature was held constant at 60°C. The measurements were carried out on a pregelatinized 20% oatmeal suspension with 0.2% Fungamyl added. Other conditions were the same as those used in viscosity measurements.

RESULTS & DISCUSSION

DSC measurements

The initial gelatinization temperature, T_i, and the gelatinization temperature, T_g, of oatmeal suspensions were determined to be 53.2 ± 0.8 and 60.1 ± 0.6°C, respectively, in the DSC measurements. Published values are: gelatinization and pasting temperatures varying between 53–72°C for pure oat starches (Gudmundsson and Eliasson, 1989; Paton, 1977; Lineback 1984). According to Donovan (1979), the gelatinization temperature is defined as the phase transition from order to disorder in the starch granule. This phase transition is endothermic and the heat uptake is registered during DSC measurements. On the other hand, the pasting temperature can be defined as the temperature at which the flow properties of the starch suspension change drastically as the suspension becomes a dispersion of swollen granules in a continuous matrix of predominantly amylose (Zobel, 1984). The initial pasting temperature registered in an Amylograph (Brabender) is generally somewhat higher than the gelatinization temperature registered by DSC. The gelatinization temperature can also differ between pure starches and flour, as has been shown for wheat (Eliasson, 1980). Both the protein content and the rather high fat level in oatmeal can influence gelatinization temperature. The amount of damaged starch is also of importance (Stevens, 1971). Differences in water content between the DSC samples, 75% (w/w) water and rheological analyses, 80–85% (w/w), should not have affected the results (Eliasson, 1980; Donovan, 1979).

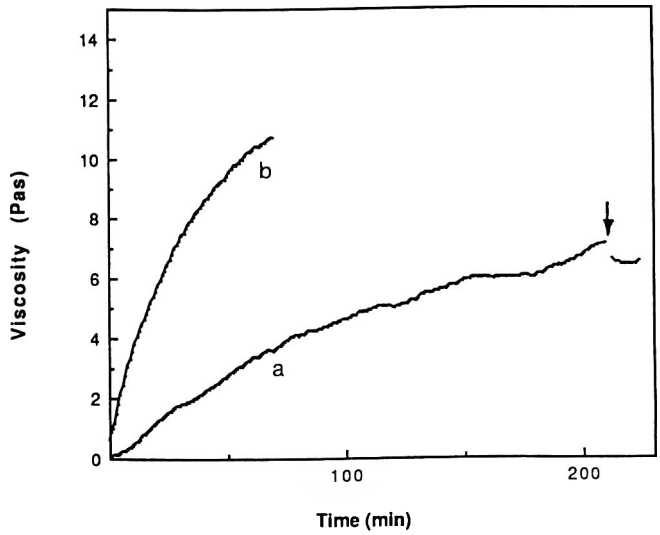

Fig. 1—Change in viscosity with time in a 20% oatmeal suspension at (a) 22°C and (b) 50°C with a constant shear rate of 1.72 sec^{-1}. Arrow indicates break in measurement where sample was carefully resuspended.

The influence of pregelatinized starch must also be considered when the oatmeal is prepared from oat flakes. Several different methods can be used to analyze the amount of pregelatinized starch. However, the DSC measurements seem to give the highest values for pregelatinized starch in a comparative study of analysis methods (Mahnke et al., 1989). Analysis of relative gelatinization enthalpy of the oatmeal compared to untreated oat kernels indicated a pregelatinization of 20% starch. This value has also been reported in DSC measurements of commercially prepared oat flakes by Mahnke et al. (1989).

Rheological measurements

Viscosity measurements below pasting temperature. The aim of the experiment was to observe any gradual decrease in viscosity with time which would indicate sedimentation. The enzymes, amylase and glucanase, that might interfere with such an observation were inactivated during the preserving heat treatment of the flour. The results from the measurements are shown in Fig. 1. At 22°C a slow increase in viscosity with time was registered. After 3½ hr the rheometer was stopped for 4 min and the sample was carefully resuspended. After resuspension the viscosity continued to increase very slowly, 3% during 15 min. The increase in viscosity was hypothesized to have two causes: early swelling of pregelatinized starch, and leakage and swelling of β-D-glucans. At 50°C the slope of the curve was higher. This could be explained by an increased leakage of β-glucans at increased temperatures (Wood et al., 1978). At 50°C, "cold swelling" of the damaged starch granules could also be of importance (Lindahl, 1989). Since no viscosity decrease with time was observed and no sudden viscosity increase occurred after resuspension we concluded that sedimentation did not occur.

Flow behavior. The shear sweep was carried out at 60°C on a pregelatinized 20% oatmeal suspension treated with 0.2% Fungamyl. This sample was chosen for two reasons. The Fungamyl enzyme was completely deactivated during the pasting cycle and could not interfere with the results. Second, with 0.2% Fungamyl enzyme added the peak viscosity was reduced

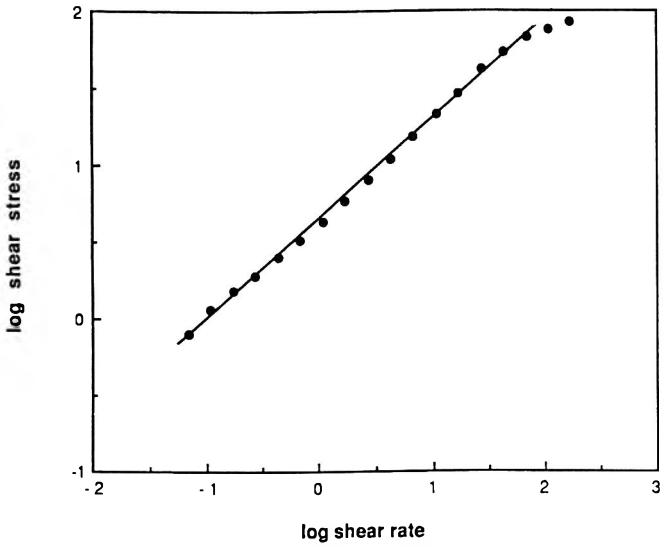

Fig. 2—Log-log plot of shear stress as a function of shear rate of a pregelatinized 20% oatmeal suspension treated with 0.2% Fungamyl at 60°C. The shear rate varied between 6.86 · 10^{-2} sec^{-1} and 1.72 · 10^{2} sec^{-1}.

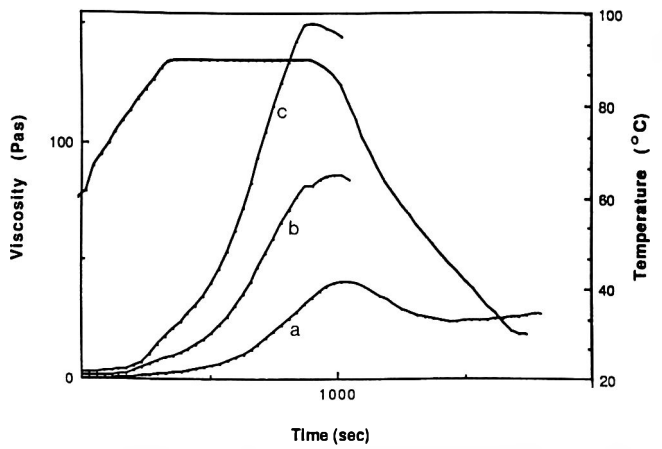

Fig. 3—Pasting behavior of (a) 15%, (b) 17.5%, (c) 20% oatmeal suspension during heating cycle from 60 to 90°C, constant temperature 10 min, then cooling.

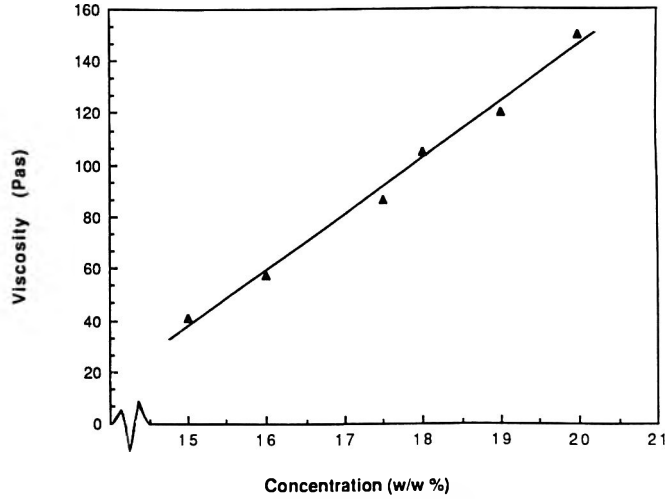

Fig. 4—Changes in peak viscosity with oatmeal concentration. Concentration interval = 15–20% oatmeal.

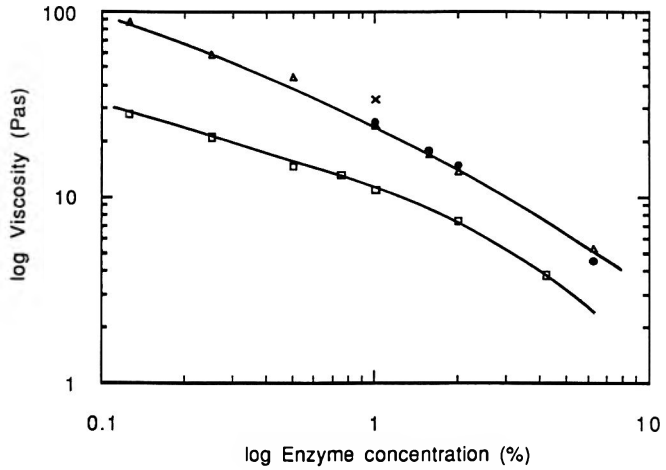

Fig. 5—A log-log plot of the peak viscosity as a function of increasing enzyme content in a 20% oatmeal suspension. ● Fungamyl added before preheating; △ BAN added after preheating; □ Fungamyl added after preheating; × 0.1% BAN heated for 10 min at 95°C.

over 90%. Larger enzyme additions caused only minor changes in peak value. The suspension had a pseudoplastic behavior, which can be seen in Fig. 2, where the log-log relationship between the shear stress, τ, as a function of the shear rate, $\dot{\gamma}$, is illustrated. When $\dot{\gamma}$ is between 0.069 and 68.68 sec^{-1} the curve is well described ($r^2 > 0.99$) by the power law equation

$$\tau = K \cdot \dot{\gamma}^n$$

where the material constant K = 0.65 and the power law coefficient n = 0.65. When $\dot{\gamma} > 68.6$ sec^{-1} there is a flattening of the curve, which may be caused by wall slip.

Pasting behavior for pure oatmeal suspensions. The pasting behavior for 15, 17.5 and 20% oatmeal suspensions is shown in Fig. 3. The viscosity peak occurred after 17 min. Increased concentration gave an earlier rise in viscosity and higher peak values. The peak values indicated that a majority of the starch granules had gelatinized. A susceptibility to shear and a development of high set-back values during cooling has been reported for many pure oat starch pastes (Paton, 1977; MacArthur and D'Appolonia, 1979). This could not be verified with the measuring technique used. All samples in the range 15–20% oatmeal had similar profiles, and differed only in peak value (Fig. 4). The relationship between peak value and oatmeal concentration was near linear in the given range ($R^2 > 0.99$). This indicated predictable behavior of suspensions of this particular oatmeal in that concentration range. The gelatinization process and the resulting gel formation were independent of the true water content but also indicated limitations for practical purposes with fast increasing viscosity values.

Addition of amylases. Figure 5 shows the dependence of peak viscosity values on the amount of amylase (BAN 120L or Fungamyl 800L) added to a 20% oatmeal suspension. Whether the addition of Fungamyl 800L was done before or after the oatmeal suspensions were heated did not affect the peak values. This was due to the fact that the preheating temperature was 50°C, i.e. below the initial gelatinization temperature of the native starch. However, if the degree of pregelatinization, measured by DSC, was as much as 20%, then differences in initial viscosity values would occur. This could not be confirmed. The ungelatinized starch granules were inaccessible to the Fungamyl amylase. Undamaged starch granules must be exposed to an amylase for a few hours before any hydrolytic effects are observed (Kuracina et al., 1987). The activity experiment was carried out because BAN is a thermostable amylase. This could be a disadvantage, as residual activity after the heat treatment would allow hydrolysis to continue. How-

Table 1—Carbohydrates, percent of dry substance in enzyme treated oat meal suspensions

	Enzyme (%)	Saccharose (%)	Maltose (%)	Raffinose (%)	Stachiose (%)	Glucose (%)	Fructose (%)
Untreated suspension	–	1.3	*	0.3	0.1	*	*
BAN 120L	0.025	0.9	*	0.3	trace	*	*
BAN 120L	0.050	1.1	*	0.3	0.1	*	*
BAN 120L	0.100	1.1	*	0.3	trace	*	*
BAN 120L	0.420	1.2	0.7	0.4	0.2	*	*
Fungamyl 800L	0.100	1.3	3.4	0.6	0.2	0.1	0.03
Fungamyl 800L	0.160	1.2	5.0	0.6	0.2	0.2	0.03
Fungamyl 800L	0.640	1.1	11.4	0.7	trace	0.5	0.02

* Below detectable limit

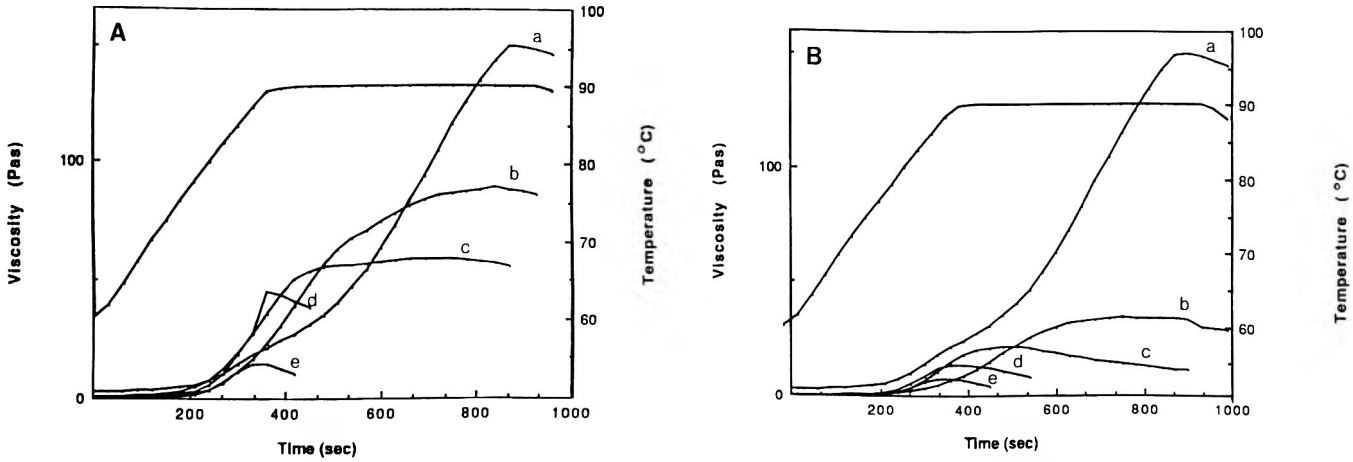

Fig. 6—Pasting behavior of a 20% oatmeal suspension with enzyme added. The samples were heated 6°C/min from 60°C to 90°C, held at constant temperature for 10 min, and then cooled. (A) Fungamyl additions: (a) 0%, (b) 0.025%, (c) 0.050%, (d) 0.075%, (e) 0.2%. (B) BAN additions: (a) 0%, (b) 0.1% heated for 10 min, (c) 0.05%, (d) 0.1%, (e) 0.42%.

ever, heat treated 0.1% BAN enzyme solution had a residual activity comparable to less than 0.01% native BAN enzyme.

At small additions of enzyme much greater reductions in peak values for the BAN treated samples than for the Fungamyl treated ones were observed. This was due to their different temperature optima and also to differences in behavior. They were both endoamylases and hydrolyze 4-α-glucosidic linkages in amylose and amylopectin, but they give different end-products (Table 1). The BAN enzyme hydrolyzes randomly and causes rapid reduction in the viscosity of the gelatinized starch. The end-products are mainly dextrins of varying chain lengths. The Fungamyl enzyme appears to work more specifically, since it does not give rise to rapid reduction in viscosity. The end-products contain large amounts of maltose and small amounts of glucose and fructose. An interesting observance was that the oatmeal suspensions treated with small additions of Fungamyl (0.025-0.075%) had an earlier increase in viscosity with increasing addition relative to the untreated sample (Fig 6a).This was not the case with the BAN enzyme (Fig. 6b). This indicated that small additions of the Fungamyl enzyme facilitated the gelation process. When the starch granules are heated, they start to swell and the amylopectin crystallite structure starts to break up. The liberated side chains of amylopectin become hydrated and start to swell (French, 1984). The Fungamyl enzymes might then hydrolyze these side chains and facilitate diffusion of amylose out of the granule and water diffusion into the granule. This could explain the early viscosity rise.

Oscillation test. The oscillation measurement in Fig. 7 shows the continuous increase of viscosity in an oatmeal suspension during heat treatment. The strains used were probably not destructive to the starch gel in the oatmeal suspension which resulted in a much higher peak value for viscosity, 800 Pas. This, in combination with a test volume of 15 mL (half the volume used in viscosity measurements), gave an earlier maximum value for the gelatinization peak. However, the most

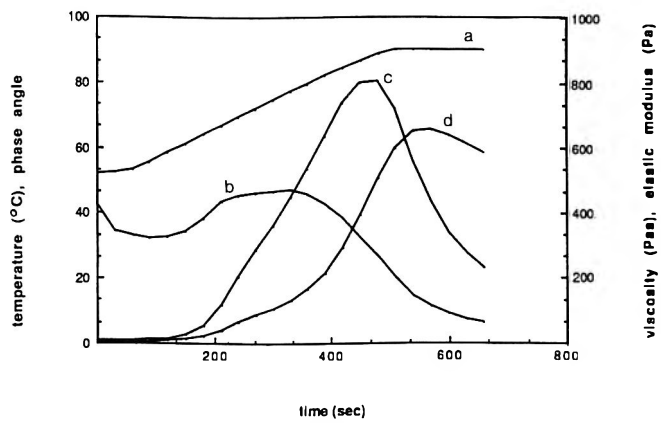

Fig. 7—Oscillation measurements performed on a 20% oatmeal suspension. Changes in temperature (a), phase angle (b), viscosity (c), and elastic modulus (d) are plotted versus *time. The temperature was increased from 52°C to 90°C and held at 90°C for 10 min.*

valuable information during the gelatinization process was derived from values of the phase angle. The initial phase angle value of about 35° was in agreement with a slightly overmixed bread dough (Lindahl and Eliasson, 1991). But in our system no gluten proteins were available, and continuous viscoelastic gluten phase as occurs in wheat flour could not form. However, the water hydration capacity of the oat proteins was similar or even greater than that of wheat gluten (Quinn and Paton, 1979). The continuous phase in the oatmeal was therefore dependent on this water-binding capacity in the proteins, combined with the immediate swelling of pregelatinized starch. It also depended on swelling of the β-glucans, and to a lesser extent on the cold-swelling of damaged starch at the initial temperature of 52°C. When the gelatinization really begins,

there is an increase of 10 degrees in phase angle. This indicates a lower ordered structure which is a result of the temporary disorder occurring in the first minutes of gelatinization. The peak viscosity indicates gelatinization of a majority of the starch granules, which leads to a new and more rigid structure in the system. Phase angle values below 10 degrees at 90°C confirm this explanation.

CONCLUSIONS

RHEOLOGICAL EXPERIMENTS have been carried out on whole flour instead of pure starch. This is of interest for industrial applications where whole flour is part of the production of different foodstuffs such as gruels for infants and adults. Complete pasting behavior of 15–20% oatmeal suspensions could be followed in the rheometer. Sedimentation, usually a problem in fundamental rheological measurements of pure ungelatinized starch suspensions, did not occur in spite of low shear rates. Very likely the hydrocolloidal properties of the β-D-glucans together with the pregelatinized starch were important in this. These properties could be very useful in further rheological characterization of starch suspensions during gelatinization. How peak viscosity is affected by changes in concentration during gelatinization of the oatmeal suspensions is of industrial importance. In pumping, drying or storing of products it is of economical interest to have the highest concentration possible. Viscosity is a limiting factor. Treatment with amylases to lower viscosity makes it possible to increase the concentrations. Different enzymes give different results in viscosity and sweetness, enabling production of several different products for use as soups, grains, gruels, drinks, etc.

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Viability and Performance of Pure Yeast Strains in Frozen Wheat Dough

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ABSTRACT

Freezing and subsequent storage of wheat doughs containing *Saccharomyces cerevisiae, S. fructuum* or *Candida boidinii* after undergoing a bulk fermentation step, reduced the viability of yeasts, depending on the species. Freezing and storage of doughs without bulk fermentation had no effect on viability. Gassing power was affected most by frozen storage, depending on storage time and species. Changes in acidification capability were small, and only significant for total titratable acidity. Changes in sugars and leavening were closely related. Results showed that the use of less active yeast species (*S. fructuum*), with high freezing tolerance in frozen dough processes would be of practical interest.

Key Words: Yeast, strains, wheat, dough, viability, freezing

INTRODUCTION

TRADITIONALLY, breadmaking has been a time-consuming process, in which most of the work must be done at night. This fact, along with need for lower labor costs and greater market areas, has led bakers to use new technologies and develop new breadmaking methods. Lately numerous procedures for refrigerating or freezing bread doughs have been developed and become widespread to other baked goods in many countries. However, the application of low temperatures in doughmaking presents problems, many of which remain unsolved. Usually, frozen doughs show variable performance after thawing. Considerable effort has been made to establish quality parameters affecting frozen doughs. Important advances have been attained through a careful study of dough formulation, yeast characteristics, fermentation period before freezing, and freezing-thawing rates. In practice, most of these parameters are interrelated and their importance is not fully understood (Wolt and D'Appolonia, 1984).

Yeast is a very widely studied subject (Holmes and Hoseney, 1984; Taguchi et al., 1975; Hino et al., 1987). How to maintain the viability and gassing power of frozen yeasts has proved to be the most important problem in the freezing of dough. Both yeast cell viability and fermentative activity are drastically affected by the freezing-thawing cycle (Hsu et al., 1979; Maitre and Calvel, 1985), the overall effect depending on the species and strains of yeast (Oda et al., 1986; Hino et al., 1987).

Several factors influence yeast performance in frozen doughs, such as cell age, protein and sugar contents of cells and yeast stability. There is disagreement concerning the effect of cell age on freezing damage in frozen doughs (Maitre and Calvel, 1985; Lloyd, 1980). Some authors recommended freezing (−150°C) (Nourigeon, 1983) or refrigerating (+4°C) (Tanaka et al., 1980; Hsu et al., 1979) the yeast before its addition to the dough. Those treatments increased freeze-tolerance of yeasts. Prestoring the yeast at +4°C delays yeast budding, preventing growth and decreasing sensitivity to freezing. Protein and sugar contents of yeast cells appear to be directly related to gassing power and viability, respectively (Hsu et al., 1979; Oda et al., 1986; Tanaka et al., 1980). Yeast protein content over 57% was associated with good performance after freezing (Hsu et al., 1979), whereas high levels of trehalose stabilized membrane cell lipids and reduced rehydration (Oda et al., 1986; Tanaka et al., 1980).

Yeast stability in frozen doughs was inversely related to the time of active fermentation undergone by yeast before freezing (Oda et al., 1986; Hino et al., 1987; Brümmer, 1984). This is considered the most decisive factor in frozen dough stability (Hino et al., 1987; Pizzinato, 1979; Kline and Sugihara, 1969; Holmes and Hoseney, 1987). Metabolites, such as ethanol and other volatile compounds, formed during fermentation, negatively affect fermentative activity of living cells (Tanaka et al., 1976; Hsu et al., 1979). Preparation of cool doughs (18–20°C), addition of yeast at the end of mixing, a reduction of proofing and/or an increase in yeast amount (5–6% flour basis) are some feasible solutions (Maitre and Calvel, 1985; Brümmer, 1984; Nourigeon, 1983).

As a result of the low ability of commercial baker's yeast to withstand subfreezing temperatures, studies aimed at development of new freeze-tolerant yeast strains for breadmaking have been reported (Nakatomi et al., 1985; Uno et al., 1987; Oda et al., 1986; Hino et al., 1987). The objective of our study was to determine the influence of dough freezing and storage time at freezing temperatures on viability and functional properties (fermentative and biochemical characteristics) of three species of yeasts isolated from wheat sourdoughs. It also provided useful information about the potential use of yeast strains other than *S. cerevisiae* for frozen doughs.

MATERIALS & METHODS

THREE STRAINS of pure yeasts: *Candida boidinii* (strain P-40), *Saccharomyces cerevisiae* (P-2), and *S. fructuum* (P-7) isolated from wheat doughs (Barber et al., 1983; Barber and Báguena, 1988) were used. Details referring to microbial mass production and preservation have been described by Barber et al. (1989). Each yeast was individually used for making wheat doughs. The basic formula of the dough consisted of 100g wheat flour (moisture, 15.45%; water absorption (farinograph), 52.9%; specific deformation work (alveograph), 135 ($\times 10^3$ ergs); P/L (tenacity/extensibility ratio (alveograph)), 0.31); 54.6 mL water; 1g glucose; 2g salt and 4g yeast (75% moisture). One kg flour was mixed in a Hobart mixer (n-50, ML-337) for 18 min. Dough temperature, after mixing, was 25 ± 1°C.

A portion of each dough was divided into 30 g pieces and frozen at −18°C (NBFD). The remaining dough was allowed to ferment (bulk fermentation) in a fermentation cabinet (National Mfg, Co. Lincoln, NE, mod. 505/55/2/3) at 28°C and 85% relative humidity (RH) to a volume increase (Δ V) of 1.8 its original size. Then it was divided into 30 g pieces and frozen as described above (BFD). Unfrozen doughs (UFD) were prepared in the same manner and used as control. Frozen doughs were stored at −18°C for periods of 1, 7 and 30 days, after which samples were thawed in a proofing chamber (28°C, 75–85% RH) until they reached a temperature of 25°C in the center of the piece. Thawed and unfrozen doughs were assayed for viability and fermentative activity.

Viable cell counts

Viable yeast cells were determined just before freezing and after thawing by the plate count method with Sabouraud dextrose agar (Har-

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Table 1—Effect of freezing and storage at −18°C on the viability (× 10^8 cfu/g dough ww) of pure yeasts

Yeasts	Frozen storage (days)	Bulk fermentation	
		Without (NBFD)[a]	With (BFD)[a]
S. fructuum	0 (UFD)[a]	4.85 ± 0.21[b]	13.00 ± 0.87
	1	8.86 ± 0.31	40.10 ± 2.04
	7	2.30 ± 1.30	13.80 ± 2.01
	30	11.20 ± 0.21	1.04 ± 0.17
S. cerevisiae	0 (UFD)	1.54 ± 0.28	15.40 ± 3.99
	1	1.83 ± 0.19	4.13 ± 1.52
	7	0.75 ± 0.11	1.30 ± 0.17
	30	1.22 ± 0.07	0.21 ± 0.01
C. boininii	0 (UFD)	1.82 ± 0.32	1.52 ± 0.12
	1	0.17 ± 0.05	0.46 ± 0.03
	7	5.40 ± 0.56	0.44 ± 0.01
	30	1.19 ± 0.06	0.46 ± 0.03

[a] UFD = unfrozen dough; NBFD = dough without bulk fermentation; BFD = dough after 1 hr bulk fermentation.
[b] standard deviation.

rigan and McCance, 1979). Plates were incubated at 30°C and colonies counted after 72 hr.

Fermentative activity

Gassing power, pH and total titratable acidity (TTA) evaluations were carried out by previously described procedures (Barber et al., 1983; 1988) and recorded during 3 hr.

Acetic acid

Acetic acid contents of doughs were measured by enzymatic methods as reported by Martínez-Anaya et al. (1989). Reagents for enzymatic analysis were purchased from Boehringer Mannheim S.A. Biochemicals, Barcelona, España.

Chromatographic analysis of sugars

Mono- and disaccharides were determined as methylsilyl derivatives by capillary gas chromatography (Martínez-Anaya et al., 1989). Chemicals used were from Sigma Chemical Co., St. Louis, Mo.

Statistical analysis

Statistical data analysis was carried out on a Microvax II (VMS/VAX) computer (Digital Equipment Co., Maynard, MA) by using the statistical package BMDP (analysis of variance 7D, 2V). Interactions among operative factors were determined.

RESULTS & DISCUSSION

Yeast cell viability

Freshly mixed UFD gave viable cell counts of similar magnitude for the three yeasts. Values ranged from 1.54 × 10^8 colony forming units (CFU)/g dough wet weight (ww) for *S. cerevisiae* to 4.85 × 10^8 CFU/g for *S. fructuum* (Table 1). Freezing and storage of doughs at −18°C without bulk fermentation (NBFD) for 30 days did not significantly influence the viability of yeasts. Viable cell counts after thawing were of the same order as those of UFD (Table 1).

During bulk dough fermentation up to 1.8 times its volume, yeast cell number increased ($P<0.05$) to 1.3 × 10^9 CFU/g and 1.54 × 10^9 CFU/g for *S. fructuum* and *S. cerevisiae*, respectively, whereas *C. boidinii* did not propagate (Table 1). Freezing and subsequent storage of BFD resulted in a decrease in survival of yeast, which depended on the species. *C. boidinii* was more sensitive to the freezing process than to storage; the effect was evident after 1 day storage, and no further changes were noticeable up to 30 days. *S. fructuum* was not influenced by freezing nor storage time up to 7 days; after 30 days a

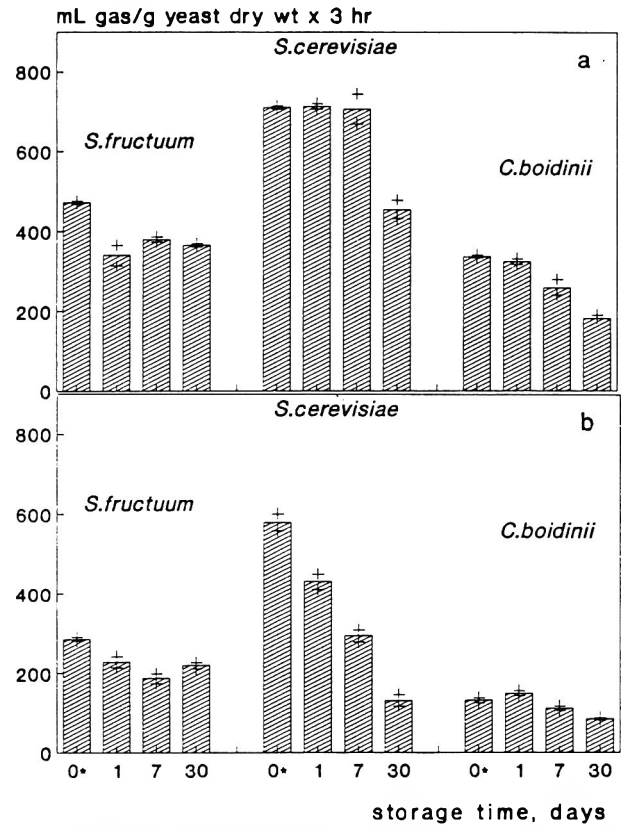

Fig. 1—Effect of dough freezing and storage at −18°C on gassing power during fermentation of doughs made with pure yeasts; (a) doughs frozen without bulk fermentation (NBFD); (b) doughs frozen after bulk fermentation (BFD); 0: unfrozen dough (UFD). (+ − + = deviation from mean).*

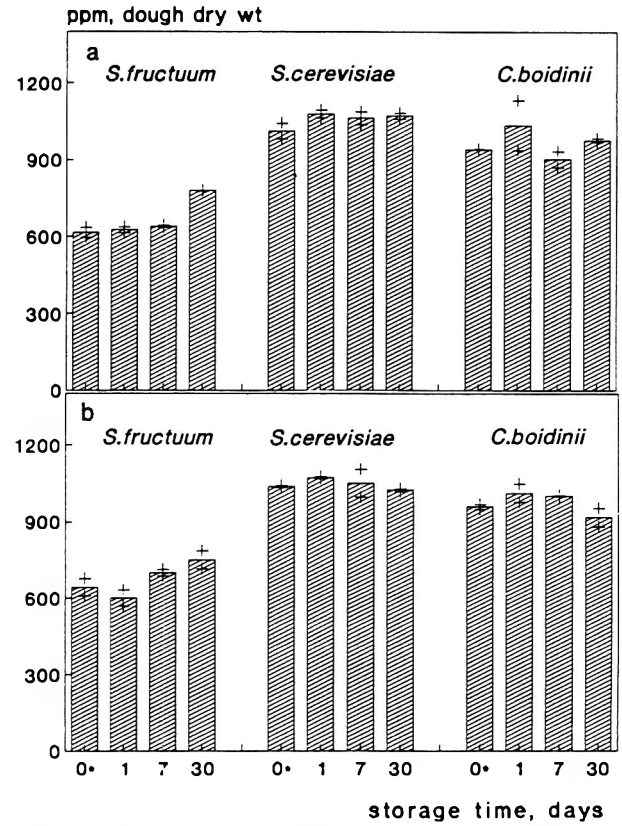

Fig. 2—Effect of dough freezing and storage at −18°C on acetic acid contents after fermentation of doughs made with pure yeasts; (a) doughs frozen without bulk fermentation (NBFD); (b) doughs frozen after bulk fermentation (BFD); 0: unfrozen dough (UFD). (+ − + = deviation from mean).*

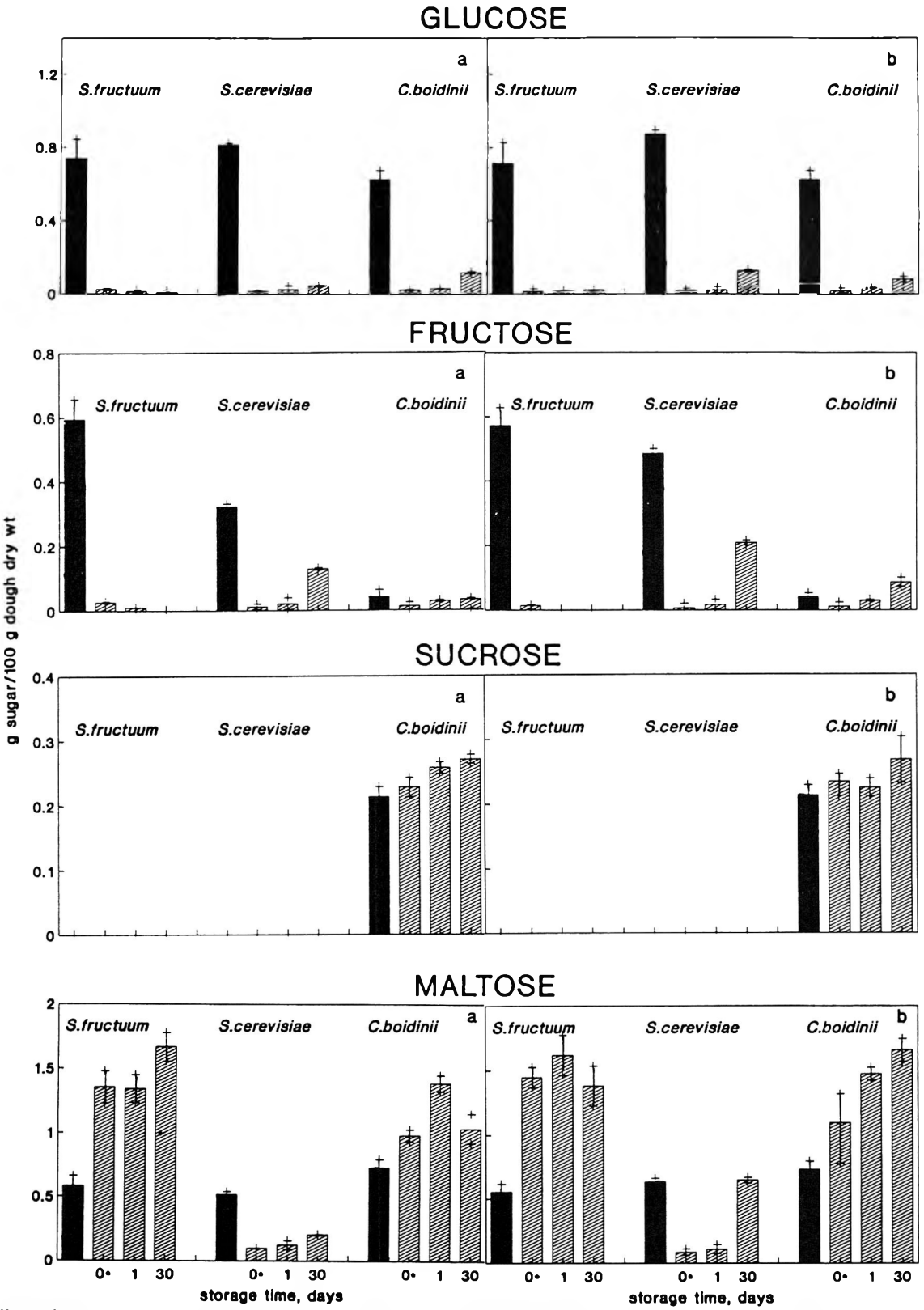

Fig. 3—Effect of dough freezing and storage at −18°C on the evolution of sugars during fermentation of doughs made with pure yeasts; (a) doughs frozen without bulk fermentation (NBFD); (b) doughs frozen after bulk fermentation (BFD); 0: unfrozen dough (UFD). (+ − + = deviation from mean).*

decrease ($P<0.05$) in viability was noticed. Viability of *S. cerevisiae* was affected ($P<0.05$) by both the freezing process and storage time. After 30 days storage, survival rates were 2.12×10^7 CFU/g. This confirmed the importance of dough fermentation before freezing on yeast viability as pointed out by others (Hino et al., 1987; Wolt and D'Appolonia, 1984; Godking and Cathcard, 1949). However, the effect was dependent on the yeast species.

Table 2—Influence of dough freezing and storage at −18°C on pH[a] developed during fermentation of doughs made with pure yeasts

Yeasts	Frozen storage (days)	Bulk fermentation			
		Without (NBFD)[b]		With (BFD)[b]	
		pH 1	pH 2	pH 1	pH 2
S. fructuum	0 (UFD)[b]	5.56 ± 0.06[c]	5.40 ± 0.03	5.61 ± 0.02	5.43 ± 0.02
	1	5.55 ± 0.03	5.47 ± 0.03	4.49 ± 0.05	5.36 ± 0.01
	7	5.72 ± 0.02	5.56 ± 0.03	5.47 ± 0.02	5.48 ± 0.01
	30	5.59 ± 0.03	5.39 ± 0.03	5.62 ± 0.04	5.53 ± 0.02
S. cerevisiae	0 (UFD)	5.44 ± 0.03	5.20 ± 0.02	5.27 ± 0.01	5.29 ± 0.04
	1	5.31 ± 0.01	5.25 ± 0.01	5.40 ± 0.04	5.21 ± 0.01
	7	5.48 ± 0.01	5.21 ± 0.01	5.40 ± 0.04	5.21 ± 0.03
	30	5.47 ± 0.02	5.30 ± 0.01	5.33 ± 0.03	5.25 ± 0.01
C. boininii	0 (UFD)	5.76 ± 0.05	5.35 ± 0.03	5.43 ± 0.02	5.39 ± 0.01
	1	5.63 ± 0.04	5.45 ± 0.01	5.47 ± 0.01	5.51 ± 0.01
	7	5.70 ± 0.02	5.58 ± 0.02	5.54 ± 0.02	5.44 ± 0.02
	30	5.70 ± 0.02	5.58 ± 0.01	5.55 ± 0.01	5.50 ± 0.02

[a] pH 1 = immediately after thawing; pH 2 = after 3 hr fermentation at 30°C and 85% RH.
[b] UFD = unfrozen dough; NBFD = dough without bulk fermentation; BFD = dough after 1 hr bulk fermentation.
[c] Standard deviation.

Table 3—Influence of dough freezing and storage at −18°C on total titratable acidity[a,b] (TTA) developed during fermentation of doughs made with pure yeasts

Yeasts	Frozen storage (days)	Bulk fermentation			
		Without (NBFD)[3]		With (BFD)[3]	
		TTA1	TTA2	TTA1	TTA2
S. fructuum	0 (UFD)[c]	5.24 ± 0.24[d]	4.50 ± 0.08	4.62 ± 0.51	3.21 ± 0.11
	1	4.70 ± 0.34	4.47 ± 0.26	3.80 ± 0.08	3.44 ± 0.05
	7	4.19 ± 0.12	3.65 ± 0.18	3.74 ± 0.34	3.58 ± 0.33
	30	3.28 ± 0.12	3.18 ± 0.12	3.84 ± 0.32	3.99 ± 0.16
S. cerevisiae	0 (UFD)	4.34 ± 0.09	3.65 ± 0.25	4.54 ± 0.27	3.45 ± 0.06
	1	4.69 ± 0.08	3.84 ± 0.10	4.25 ± 0.03	3.96 ± 0.10
	7	5.16 ± 0.15	3.98 ± 0.29	3.88 ± 0.10	4.40 ± 0.08
	30	3.85 ± 0.16	4.48 ± 0.08	4.33 ± 0.15	4.01 ± 0.19
C. boininii	0 (UFD)	4.67 ± 0.17	4.81 ± 0.61	4.11 ± 0.20	4.38 ± 0.21
	1	4.40 ± 0.39	4.51 ± 0.08	4.11 ± 0.08	4.32 ± 0.11
	7	4.93 ± 0.67	4.30 ± 0.18	4.56 ± 0.16	4.39 ± 0.23
	30	5.82 ± 0.27	3.99 ± 0.20	3.90 ± 0.08	3.92 ± 0.12

[a] mL 0.01 N NaOH/100 g dough ww.
[b] TTA1 = immediately after thawing; TTA2 = after 3 hr fermentation at 30°C and 85% RH.
[c] UFD = unfrozen dough; NBFD = dough without bulk fermentation; BFD = dough after 1 hr bulk fermentation.
[d] standard deviation.

Fermentative activity

Gassing power. As reported previously (Martínez-Anaya et al., 1989), *S. cerevisiae* was the most active yeast in UFD followed by *S. fructuum* and *C. boidinii*. Gas production after 3 hr fermentation was 710 mL CO_2/g dry yeast, 473 mL and 337 mL, respectively, in UFD without bulk fermentation (Fig. 1a). UFD which underwent a bulk fermentation step gave lower gas volumes after 3 hr, but relative amounts produced by each yeast were maintained (Fig. 1b). Those doughs underwent a longer total fermentation period (4–5 hr) than the former (3.5 hr), and gas production decreased ($P<0.05$) mainly because of a lack of directly fermentable sugars (Fig. 3).

The freezing of NBFD caused a decrease ($P<0.05$) of 28% in gas production after 3 hr fermentation for thawed dough prepared with *S. fructuum* but not with *S. cerevisiae* nor *C. boidinii* (Fig. 1a). Storage at −18°C up to 30 days did not result in further changes with *S. fructuum* but with the other two yeasts different trends were observed. *C. boidinii* showed a continuous loss of gassing power as storage time increased. Gas production ability of *S. cerevisiae* was not affected during short storage periods (7 days), but a drastic decrease (36%) was detected after 30 days storage (Fig. 1a).

When BFD was defrosted, the total gas produced after 3 hr fermentation was considerably lower than that of NBFD (Fig. 1a,b). *S. cerevisiae* was the species most stressed by bulk fermentation prior to freezing (Fig. 1b). *S. fructuum* produced the highest amount of CO_2 after 30 days of frozen storage. The loss of viability found for *S. cerevisiae* in BFD (Table 1) after 30 days storage could be responsible for the weakening of gassing power. However, other factors such as partial cell damage and interference with metabolic mechanisms should also result in the yeast being unable to ferment sugars. This would affect its leavening ability during long storage times in NBFD.

Changes in pH and TTA. Changes in the pH of UFD during 3 hr fermentation were small for both doughs with and without a bulk fermentation stage for the three species tested (Table 2). *S. cerevisiae* reached the lowest pH (5.20) after fermentation, but *C. boidinii* underwent the greatest decrease (0.41) in the same time. Freezing and the subsequent storage of doughs did not have, in general, any effect ($P<0.05$) on the pH developed by thawed doughs during fermentation (NBFD and BFD).

Larger differences were detected in the TTA of doughs after freezing treatment. Although the magnitude of changes was small (Table 3), the differences were significant ($P<0.05$) in many cases. Yeasts showed different behaviour: *S. cerevisiae* tended to increase TTA after fermentation with the freezing and storage of doughs, in both samples, NBFD and BFD. *C. boidinii* showed an opposite tendency, and TTA decreased after treatments. Finally, for *S. fructuum* TTA was different depending on the degree of fermentation of doughs before freezing (Table 3). However, the overall practical significance of those changes was very small.

Biochemical Characteristics

Production of acetic acid. As small amounts of lactic acid were produced by the yeasts under study (Martínez-Anaya et al., 1989), only the production of acetic acid was measured.

Levels of this acid after fermentation (3 hr) of UFD varied with the species. Doughs with *S. cerevisiae* contained 1012–1038 ppm (dry wt.) of acetic acid, *C. boidinii*, 941–959 ppm, and *S. fructuum*, 616–644 ppm, when unfermented and bulk fermented doughs, respectively, were considered (Fig. 2). This agreed with the findings reported by Martínez-Anaya et al. (1989). Neither the freezing process nor the length of frozen storage modified the ability of *S. cerevisiae* to produce acetic acid during 3 hr fermentation (Fig. 2) of NBFD and BFD. *C. boidinii* and *S. fructuum* had a tendency to give slightly higher amounts of acetic acid after freezing, although this varied with period of storage. However, differences in acetic acid production between UFD and frozen doughs (NBFD and BFD) never exceeded 10%, and were not, in general, significant. These results were in agreement with those for pH and TTA of doughs.

Changes in individual sugars. Glucose and fructose levels in freshly mixed UFD made with *S. fructuum* or *S. cerevisiae* were higher (0.74–0.81g glucose/100g dough dry wt, and 0.52–0.32% fructose, respectively) than those of doughs prepared with *C. boidinii* (0.62 and 0.04%) (Fig. 3). Flour contains low amounts of these sugars (Magoffin and Hoseney, 1974; Pomeranz and Finney, 1975), so different hydrolytic activities of *S. cerevisiae* and *S. fructuum* invertases on sucrose, raffinose and/or glucofructans from flour (Pomeranz and Finney, 1975; Biltcliffe, 1972; Nilsson et al., 1987) seemed the most probable source of differences. The higher level of glucose came from the glucose included in dough formulation. Sucrose was not detected in doughs with *S. cerevisiae* or *S. fructuum. C. boidinii* is unable to ferment either sucrose or maltose (Lodder and Kreger-van Rij, 1984), which could explain the presence of sucrose after mixing (0.21%). The sucrose values coincided with those reported in flour by Pomeranz and Finney (1975), and because of the lack of hydrolysis of sucrose, the lower values found for glucose and fructose. Maltose contents of doughs ranged from 0.50% to 0.73% for the three yeasts and resulted from flour amylase action on damaged starch (Magoffin and Hoseney, 1974), which increased maltose levels up to 10–15 times its value in flour during mixing (Pomeranz and Finney, 1975).

Changes in sugar contents during 3 hr fermentation of UBD related well to CO_2 production (Fig. 2). Glucose consumption by yeast ranged from 96.4 to 98% of that found after mixing. The greatest decrease in glucose was observed with *S. cerevisiae* (Fig. 3). Fructose decreased at a lower rate than glucose (Koch et al., 1954), but after 3 hr fermentation, fructose consumption was close to 96% for *S. cerevisiae* and *S. fructuum, C. boidinii* used 68% of the fructose present after mixing.

Sucrose levels in doughs with *C. boidinii* after fermentation were similar to those detected after mixing. Maltose content decreased to about 85% of its initial value after fermentation of dough with *S. cerevisiae* but increased in doughs with the other yeasts, which did not ferment the sugar (Lodder and Kreger-van Rij, 1984). Final levels were higher for *S. fructuum* than for *C. boidinii* (1.38 and 0.97%, respectively). In general, no differences were observed in the final amounts of sugars after fermentation between UFD with and without a bulk fermentation step. Results were in agreement with those reported previously (Martínez-Anaya et al., 1989) for other strains of the same yeasts.

Freezing did not influence the levels of glucose and fructose remaining after 3 hr fermentation as compared with those observed for UFD. Storage of frozen doughs for long periods (30 days) caused a decrease ($P < 0.05$) in consumption of both sugars by *S. cerevisiae* and *C. boidinii* (Fig. 3). This could be attributed to the reduced viability and leavening activity showed by these yeasts after long frozen storage periods (Table 1 and Fig. 1). Fermentation of doughs prior to freezing (BFD) did not influence the residual content of glucose and fructose in relation to NBFD.

The amount of sucrose in fermented doughs increased after freezing (UBFD) and subsequent storage (UBFD and BFD) of frozen doughs (Fig. 3) with *C. boidinii*. This could be due to the sugar produced by hydrolysis of raffinose or glucofructans (Biltcliffe, 1972; Nilsson et al., 1987) from flour during the freezing-storing-thawing cycle and the inability of this yeast to utilize sucrose. Maltose contents of fermented doughs after freezing were also higher than those detected in UFD (Fig. 3). Differences were greater in BFD than in NBFD, specially with *C. boidinii* and *S. cerevisiae* and at long periods of storage. This was consistent with the data for viability and gassing power.

Combinations of yeasts *S. cerevisiae* and *S. fructuum* in unfrozen wheat doughs (Martínez-Anaya et al., 1990) have had a positive synergistic effect on gas production resulting from their interaction. Because of its inability to ferment maltose, *S. fructuum* will not compete with *S. cerevisiae* for maltose which during fermentation and storage of frozen doughs reached noticeable levels. Since maltose is the most important available substrate during fermentation, associations of both yeasts could offer potential interest in the making of frozen doughs without or after a bulk fermentation stage previous to freezing. More work must be done to confirm these assumptions and establish any technological advantages of using other yeast species naturally present in wheat doughs together with baker's yeast.

CONCLUSION

FREEZING and storage up to 30 days of frozen unfermented doughs did not adversely affect the survival of *S. cerevisiae*, *S. fructuum* and *C. boidinii*. Fermentation of doughs prior to freezing had a deleterious effect on viability and gassing power of *S. cerevisiae* and *C. boidinii*, whereas *S. fructuum* maintained high survival and gassing power rates.

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Optimization of Extrusion Cooking Process for Chickpea (*Cicer arietinum*, L.) Defatted Flour by Response Surface Methodology

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ABSTRACT

Response surface methodology was employed to optimize the production of a snack food from chickpea. The independent variables, process temperature (123–137°C) and feed moisture (13–27% d.s.b.) were selected at five levels (rotatable five level composite design: $-\sqrt{2}$, -1, 0, 1, $+\sqrt{2}$) in the extrusion of defatted chickpea flour. Response variables were expansion ratio, shear strength of the extrudate and sensory preference assessed by an untrained panel. Expansion ratio increased steadily with decrease in feed moisture similar to cereal extrusion. Regions of maxima were observed for sensory preference and shear strength, and these two product attributes were linearly related. The most acceptable chickpea snack was rated higher than a commercial corn snack.

INTRODUCTION

PROTEIN MALNUTRITION prevalent in several populated regions requires an increase in both dietary protein intake and/or protein biological value of foodstuffs. The high production costs and the low efficiency of protein conversion by livestock make vegetable protein more suitable for this purpose. Remarkable progress has been made in the utilization of new protein sources such as oilseed, cereal, leguminous seed, leaf and single cell proteins (Altschul, 1974; Kinsella, 1978). Legumes are an important source of food protein and other nutrients (Chavan and Salunkhe, 1986; Milner, 1974; Salunkhe, 1982). Among legumes chickpea (*Cicer arietinum*, L.) is fifth in world production (Chavan and Salunkhe, 1986). Protein contents of cultivars of this bean range from 20 to 31%. The nutritional value of chickpea protein is superior to those of soybean, cowpea, mung beans, peas, dry bean, lentils, faba bean, pigeonpea and black gram (Khan et al, 1979; Pak and Barja, 1974; Chandrasekharappa, 1979).

Chickpea, consumed mainly in Eastern countries is not very popular in Europe and the U.S. (Chavan and Salunkhe, 1986). Use of this bean in Western foods may be implemented through extrusion to produce either a functional protein food ingredient or a snack product. The high starch content of chickpea (Chavan and Salunkhe, 1986) favours extrusion to produce expanded snack products. The advantage of such a product over the traditional corn snack would be its higher protein content and superior protein nutritional quality. The objective of our work was to optimize the extrusion cooking process for production of a chickpea snack.

MATERIALS & METHODS

Materials

Chickpea (*Cicer arietinum*, L.), provided by "Instituto Agronômico de Campinas, SP", strain "IAC Marrocos" was originally a genotype from the "Service de la Recherche Agronomique et de l'Expérimentation Agricole de Robat", Morocco. The sample for extrusion was prepared by grinding the beans in a hammer mill (Mod. TP3 - Máquinas Cordeiro, Brasil) followed by defatting wth ethyl ether in a Soxhlet apparatus.

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Methods

Proximate composition. The following conventional methods were used: desiccation at 105°C, for moisture; calcination at 550°C, for ash; defatting in Soxhlet apparatus with 2:1 chloroform/methanol, for lipids; and microkjeldahl for protein (N × 6.25) (AOAC, 1980). Fibre was determined as insoluble fraction in neutral detergent (Van Soest and Wine, 1967) and carbohydrate content was estimated by the difference from 100, of the sum of percentages of moisture, ash, lipid, protein and fiber.

Expansion ratio. Expansion ratio was reported as the ratio of extruded product diameter and the diameter of the die hole. Values reported were averages of 24 measurements.

Shear strength. The force necessary to shear completely the extrudates was determined using an Instron equipment (Mod 1000 - Instron Corp. - USA) with a Warner-Bratzler device. The crosshead speed was 500mm/min and values reported were averages of 10 determinations.

Sensory preference. A nonnumeric scale of 100mm (Pangborn, 1984a; Land and Shepherd, 1988) was used by panelists to compare sensory properties of extrudates with a commercial brand of corn based snacks. The panel consisted of eight untrained persons recruited from the students and staff of our Department.

Extrusion. A single-screw extruder (Miotto Ltda, Sao Paulo, Brasil) with a length-to-diameter ratio 20:1 was employed. The barrel (smooth) was divided into three independent electrically heated zones cooled by water. Conditions during extrusion were fixed at: 200 rpm screw rotation; 4:1 screw compression ratio; 4mm die diameter. The feed end of the barrel was set at 10°C less than the central zone and the die end of the barrel was set at 20°C less than the central zone. This arrangement produced a temperature profile which started at ambient in the feed zone, followed by a rapid increase up to maximum in the second zone, and a decrease in the die. This final temperature decrease in the exit of the molten mass provided higher viscosity of the dough in the die when compared to the second zone, which prevented evaporation of superheated water before the exit of the material (Smith et al, 1982). A screw operated feed hopper fed the extruder at a feed screw speed of 20 rpm. Feed rate was 73.5 g/min. Extrusion parameters as independent variables were: temperature of the central zone of the barrel and moisture content of the feed.

Partial size of the feed was 1 mm. This was obtained by sieving to uniform particle size defatted flour which was hydrated with 4X the necessary water for the required feed moisture, dried at 65°C in fluxing air for 4h, and ground in a paddle homogenizer (Arn S/A - Brasil). The moisture content of the powder was determined, and water was added in a homogenizer (Model Planetária-Arno S/A, Brasil) to the required moisture level. The hydrated feed materials were kept in a refrigerator overnight in sealed plastic bags. Defatted soya grits (provided by Sanbra S/A, Brasil; Prosam F) with previously determined optimum moisture content for extrusion, (Barros et al., 1987) were fed until steady-state was achieved. Then the chickpea feed was introduced.

Optimization. The process was optimized for maximum values of expansion ratio and sensory ratings for extrudates; and to minimum values of shear strength. Initial experiments at feed moistures 10 to 25% (d.b.) and, central section temperature 130–160°C, showed that good expansion could be obtained at 130°C and 25% moisture. At 10% feed moisture the extruder stopped. Minimum feed moisture for proper operation of the extruder was 13%. Based on these preliminary

Table 1—Specification matrix of the second order design X_1: moisture (% d.s.b.); X_2: temperature (°C)

Assay no.[a]	Coded variables x_1 $[(X_1 - 20)/5]$	Coded variables x_2 $[(X_2 - 130)/5]$	Original variables X_1 (% M)	Original variables X_2 (T)
1	−1	−1	15	125
2	+1	−1	25	125
3	−1	+1	15	135
4	+1	+1	25	135
5	$-\sqrt{2}$	0	13	130
6	$+\sqrt{2}$	0	27	130
7	0	$-\sqrt{2}$	20	120
8	0	$+\sqrt{2}$	20	140
9	0	0	20	130
10	0	0	20	130
11	0	0	20	130
12	0	0	20	130
13	0	0	20	130

[a] Does not correspond to order of processing

Table 2—Proximate composition of chickpea flour[a]

	Moisture	Ash	Lipids	Protein	Fiber	Carbohydrate
Original flour	16.30 ±0.23	2.79 ±0.13	8.60 ±0.60	14.10 ±0.48	16.79 ±0.92	41.42 ±1.08
Defatted flour	5.74 ±0.09	3.46 ±0.50	2.90 ±0.49	20.27 ±1.50	18.37 ±0.97	49.26 ±1.86

[a] Average of three determination ± st dev, % carbohydrate by difference.

Table 3—Expansion ratio (y_1), sensory preference (y_2) and shear force (y_3) of the extrudates

Assay no.[a]	Moisture $x_1 = (M-20)/5$	Temperature $x_2 = (T-130)/5$	y_1[b]	y_2[c] (mm)	y_3[d] (N)
1	−1	−1	3.07	39.56	28.47
2	1	−1	2.43	6.50	73.48
3	−1	1	2.80	37.56	29.20
4	1	1	2.17	17.00	64.83
5	$-\sqrt{2}$	0	3.19	51.44	13.66
6	$\sqrt{2}$	0	2.01	8.31	106.72
7	0	$-\sqrt{2}$	2.67	26.75	63.06
8	0	$\sqrt{2}$	2.28	31.44	34.23
9	0	0	2.66	33.44	41.80
10	0	0	2.72	35.75	32.90
11	0	0	2.75	34.31	34.16
12	0	0	2.57	39.13	35.50
13	0	0	2.68	24.00	30.10

[a] Does not correspond to order of processing.
[b] Average of 24 determination
[c] Average of 8 panelists
[d] Average of 10 determination

experimental data, a randomly assigned five level design ($-\sqrt{2}$, -1, 0, 1, $\sqrt{2}$) (Myers, 1971; Cochran and Cox, 1957) was employed (Table 1). Results were fitted to a second order polynomial:

$$y = B_0 + B_1x_1 + B_2x_2 + B_{12}x_1x_2 + B_{11}x_1^2 + B_{22}x_2^2 + E$$

where y = dependent variable (expansion ratio, shear strength or sensory preference); x_1 = feed moisture; x_2 = temperature of the central zone of the barrel; E = experimental error with normal distribution, mean zero, and variance σ^2.

The order of processing was chosen by randomizing feed moisture levels at increasing levels of temperature. Operation started from the lowest temperature and operation continued without stopping between samples. Statistical analysis and graphics were done using the statistical package program STATGRAPHICS (Statistical Graphics Corporation. USA, STSC Inc., 1987).

RESULTS & DISCUSSION

THE PROXIMATE COMPOSITION of the chickpea flour (Table 2) was in the range of published reports (Chavan and Salunkhe, 1986). Table 3 presents results for expansion ratio, sensory scores and shear strength of extrudates. Fig. 1 shows the extrudates for all temperatures and feed moisture conditions assayed.

Response surface methodology has been successfully used for optimization of several food processes (Aguilera and Kosikowski, 1976; Oliveira and Silva, 1983; Hauly et al., 1980; Bastos and Arêas, 1990; Barros et al., 1987; Frazier et al., 1982). In extrusion cooking process, a 2nd order polynomial has accounted for more than 90% of the variation observed (Aguilera et al., 1976; Frazier et al., 1982). Table 4 shows the analysis of variance of the response variable expansion ratio. The results indicated that linear effects of both moisture

Table 4—Analysis of variance for the full regression of the 2nd degree polynomial for the response: Expansion ratio (y_1)

Source	Coefficient	t-value	Significance level
Constant	2.675999	69.6552	0.0000
x_1	−0.367348	−12.0949	0.0000
x_2	−0.135194	−4.4512	0.0030
x_1^2	−0.017999	−0.5526	0.5977
x_2^2	−0.080501	−2.4716	0.0427
x_1-x_2	0.00250	0.0582	0.9552

	Sum of squares	DF	Mean square	F-Ratio	P-value
Model	1.27127	5	0.254253	34.4534	0.0001
Error	0.051657	7	0.0073796		

$R^2 = 0.9610$

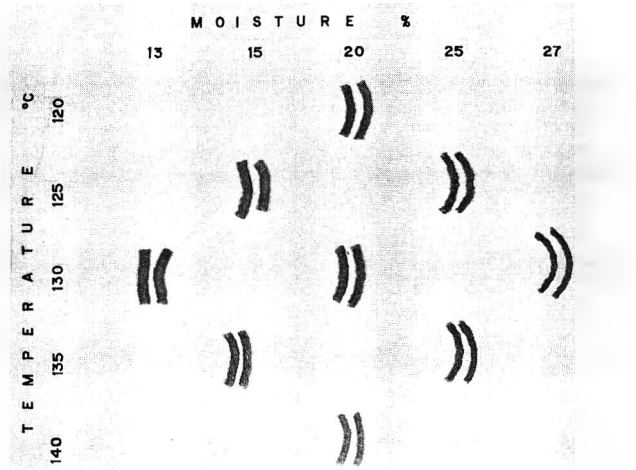

Fig. 1—Extruded chickpea in all conditions of moisture and temperature.

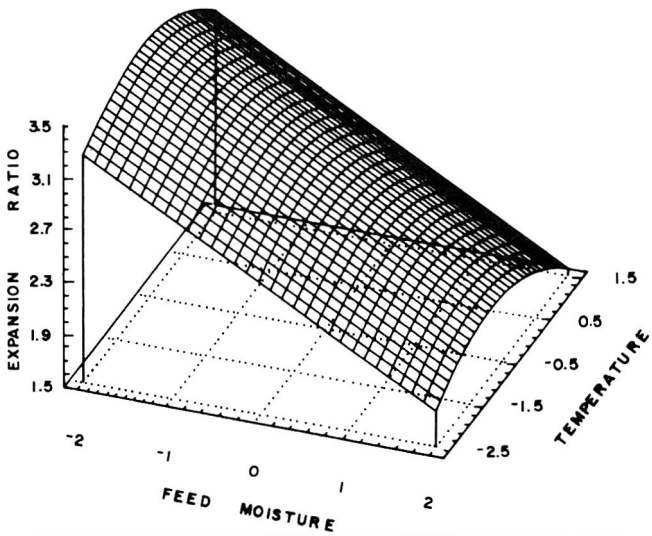

Fig. 2—Response surface for the effect of feed moisture and process temperature on expansion ratio of chickpea extrudates.

Table 5—Analysis of variance for the full regression for the 2nd degree polynomial for the response: Sensory preference (y_2)

Source	Coefficient	t-value	Significance level
Constant	33.326033	15.0224	0.0000
x_1	−14.326943	−8.1690	0.0001
x_2	−1.891593	−1.0786	0.3165
x_1^2	−2.808052	−1.4930	0.1791
x_2^2	−3.19806	−1.7004	0.1329
$x_1 \cdot x_2$	3.12500	1.2599	0.2481

	Sum of squares	DF	Mean square	F-Ratio	P-value
Model	6952.61	5	1390.52	16.3868	0.0010
Error	593.99	7	84.86		

$R^2 = 0.9213$

Table 6—Analysis of variance for the full regression for the 2nd degree polynomial for the response: Shear force (y_3)

Source	Coefficient	t-value	Significance level
Constant	34.892042	8.4697	0.0001
x_1	26.530936	8.1462	0.0001
x_2	−6.086482	−1.8688	0.1039
x_1^2	11.293477	3.2335	0.0144
x_2^2	5.520866	1.5807	0.1580
$x_1 \cdot x_2$	−2.345000	−0.5091	0.6263

	Sum of squares	DF	Mean square	F-Ratio	P-value
Model	1821.36	5	364.273	14.8036	0.0013
Error	172.25	7	24.607		

$R^2 = 0.9136$

and temperature were significant and the second order effect of temperature was significant. A resultant polynomial for this variable is:

$$y_1 = 2.676 - 0.367\, x_1 - 0.135\, x_2 - 0.081\, x_2^2$$

The response surfaces for this variable are presented in Fig. 2. It is worth noting that as moisture decreased, expansion ratio increased linearly up to the limit of moisture for the proper operation of the equipment. This behaviour is typical for extrusion of cereals and is caused by their high starch content (Guy and Horne, 1988). In our case, the high level of starch of the chickpea flour was responsible for the observed pattern. High starch fractions isolated from legumes also show good expansion. The reported temperatures of better expansion were near those observed in our work (Gujska and Khan, 1990). A possible commercial application of extruded chickpea, as with extruded cereal products, is a snack foods. In that case, the force necessary for completely shearing the sample would be important for sensory acceptance of the product (Voisey and Stanley, 1979).

The variance analysis of the 2nd degree polynomial for sensory preference and force to shear the extrudates are presented in Tables 5 and 6, respectively. Sensory preference was defined as the measure in mm of the distance from origin assigned by the panelist in a nonnumeric scale of 100mm with the central position marked as the standard product (a commercial brand of corn snacks). The panelists were instructed to consider only the texture aspect since the extruded chickpea was plain, without added flavor. Results were consistent for each panelist and reproducible in different sessions. The variance analysis of the sensory preference (Table 5) showed significant ($p < 0.05$) linear effects of moisture but not of temperature; and quadratic effects of moisture and temperature when they were near significance levels. As sensory preference always tends to a maximum or minimum when any sensory stimulus varies (Pangborn, 1984b), the quadratic coefficients were necessary to the model. In this case, those two coefficients (−2.808 and −3.198) (Table 5) were kept (acceptance level thus > 82%) due to their importance to the process and the polynomial was defined as:

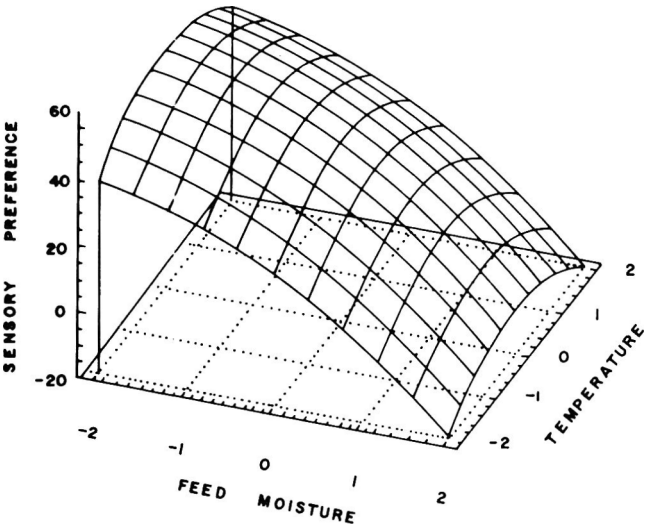

Fig. 3—Response surface for the effect of feed moisture and process temperature on sensory preference of chickpea extrudates.

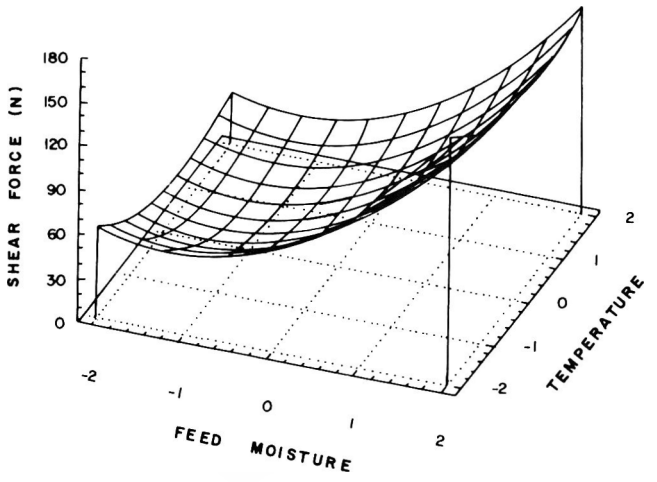

Fig. 4—Response surface for the effect of feed moisture and process temperature on force to shear the chickpea extrudates.

$$y_2 = 33.326 - 14.327\, x_1 - 2.808\, x_1^2 - 3.198\, x_2^2$$

The response surfaces of this variable are presented in Fig. 3. A region of maximum response can be seen from this plot. The partial derivatives of sensory preference in respect to moisture and temperature indicated that the conditions of processing for maximum acceptance of the product were 7.25% moisture and 130°C. However, at that moisture content the equipment was not operative and the minimum moisture usable was 13%. Maximum sensory preference of the product was 13% which corresponds to assay number 5 in Table 3 where, in fact, maximum scores were assigned by panelists, indicating higher preference than the standard.

The same significance levels (>82%) were applied to the analysis of variance of force to shear the sample (Table 6). In that case, linear and quadratic effects were significant for both moisture and temperature and the resultant polynomial was:

$$y_3 = 34.892 + 26.531\, x_1 - 6.086\, x_2 + 11.293\, x_1^2 + 5.521\, x_2^2$$

That response surface for this variable is presented in Fig.

4. A region of minimum shear force was observed and the partial derivatives showed that the independent variables for this minimum were 14% moisture and 133°C. Those conditions are near those for processing chickpea which resulted in maximum observed acceptance. This suggested that the two variables could be related. Regression analysis tests showed a linear correlation between sensory preference and force to shear the extruded sample with a high correlation level ($p < 0.00007$). For these snacks, maximum acceptance occurred in the highly expanded snacks where minimum forces were necessary to shear the product.

Our work demonstrated the high potential for use of extruded chickpea as an alternative snack food with the advantage of higher protein level compared to corn or cereal products (Chavan and Salunkhe, 1986). Moreover, nutritional quality of chickpea protein is superior to cereals or to other leguminous seeds (Chavan and Salunkhe, 1986). Preliminary assays in our laboratories for the most acceptable product (assay number 5 of Table 3) showed a very good nutritional value for the protein with PER value of 2.60, near that of casein 2.94, and digestibility coefficient of 84%.

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Sensory and Nutritional Properties of Cookies Based on Wheat-Rice-Soybean Flours Baked in a Microwave Oven

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ABSTRACT

Three formulas of cookies prepared from 50:45:5 (I), 50:40:10 (II) and 50:35:15% (III) wheat flour, broken rice flour, and defatted soy flour, respectively, baked in a microwave oven (2450 MHz) for 240 sec, were rated as the best of six formulas for flavor and texture by a trained panel. Cookies of formulas I and II were preferred over formula III by a consumer-type panel. Cookies of all three formulas showed no significant differences in nitrogen balance, apparent biological protein value and apparent net protein utilization. Apparent protein digestibility and protein efficiency ratio were significantly higher in formulas II and III than in formula I.

Key Words: cookies, nutrients, sensory evaluation, wheat flour, rice flour, defatted soy flour

INTRODUCTION

COOKIES comprise a major category of snacks by virtue of their general acceptability, convenience and long shelf life (Tsen, 1976). In many countries cookies are prepared with fortified or composite flour (Buck et al., 1987; Lorenz, 1983; Sambucetti et al., 1976). Replacing part of wheat flour with rice flour and soy flour is likely to improve the nutritive value of the product due to the complimentary nature of the amino acid profiles derived from these raw materials (Bakar and Hin, 1985; Cheryan et al., 1979; Suárez, 1958; Vargas et al., 1985). It also decreases the cost of the final product in countries where soybean and rice are abundant and wheat flour for the baking industry is imported (Akobundu et al., 1988).

Conventional baking of cookies and other flour products causes significant nutritonal losses through thermal degradation and Maillard reaction (Sambucetti et al., 1976; Tsen et al., 1977). Microwave baking may be an interesting alternative method for preventing nutrient degradation. Heating is more uniform and efficient, permitting baking the product in a much shorter time than conventional baking (Aref, 1968; Jeppson, 1964; Lorenz et al., 1973; Tsen, 1980). The objectives of our study were to determine the rheological properties of blended flours containing different proportions of wheat, rice and soybean and to evaluate the flavor and texture quality and the protein nutritive value of cookies prepared with these flours after baking in a microwave oven.

MATERIAL & METHODS

Raw materials

Commercial wheat flour, broken rice flour and defatted soy flour were used to produce blended flours. The flours were passed through a Produtest Sieve (Brazilian industry) corresponding to 20, 35, 60, 80, 100, 150 and 200 mesh to obtain the particle size distribution. Protein, fat and ash were determined according to the AACC (1969) methods and crude fiber according to Van de Kamer and Van Ginkel (1952).

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Blended flours and rheological properties

Blended flours were prepared as follow: commercial wheat flour (50%) was combined with 45:5, 40:10, 35:15, 30:20, 25:25 and 20:30% broken rice flour and defatted soy flour, respectively, and designated as formulas I, II, III, IV, V and VI in the same order. The flours were then mixed for 10 min in a planetary mixer (Arno model BPA-E).

Water absorption, arrival time, departure time, dough development, stability and mixing tolerance index were determined for the blended flours in Brabender Farinograph according to the constant flour method 54-21 of AACC (1976). Three hundred grams flour was mixed at optimum water absorption and the farinograph curve was centered on the 500 BU line.

Dough preparation

The ingredients used for preparation of the cookies were: blended flour (60%), sugar (22%), margarine (16%), salt (1%), baking powder (Royal, 1%), and water in the proportions of 9, 10, 11, 12, 13, and 14 mL/100g of formulas I, II, III, IV, V and VI, respectively. Sugar and margarine were creamed for 10 min in a planetary mixer (Arno model BPA-E). Then water was added with mixing for an additional 5 min. In the same manner, salt and baking powder were added to the blended flour and mixed for 10 min. After addition of all ingredients, mixing continued another 15 min. The prepared dough was put in polyethylene bags to rest for about 1 hr, flattened manually using a wooden roller into a sheet about 2.50 ± 0.05 mm thick, and then cut into spherical pieces measuring 3.5 cm in diameter.

Baking in microwave oven

Rectangular Pyrex glass ($22 \times 35 \times 4$ cm) was utilized for baking in the domestic Sanyo-Climax microwave oven (model EM90038). Baking times were: 180, 210, 240 and 270 sec. Baked cookies were cooled and placed in polyethylene bags until analysis.

Sensory evaluation

The cookies were initially submitted to overall sensory evaluation by a trained panel, taking into consideration the following descriptors: color, flavor, and texture. A rating scale of 1 to 9 points (1 = extremely poor; 9 = excellent) was used to select the best baking time. Samples with an overall quality score equal or superior to 7.5 (between 7 = good and 8 = very good) were again submitted to sensory evaluation for flavor and texture by the same panel, using the same 9-point scale. The trained panel consisted of 10 members selected randomly from students, laboratory staff and lecturers of the faculty. They were trained and instructed to rate the score of overall quality, flavor and texture of the cookies. The tests were conducted in individual booths in a Sensory Evaluation Laboratory. Cookies were produced 24 hr before the panel test. A consumer-type preference test was carried out with samples that showed higher scores of flavor and texture, using 75 untrained consumer-type panelists and the ranking procedure (Kahan et al., 1973).

Nutritional evaluation

Nutritional evaluation was done on the selected cookie formulas by proximate analysis (AACC, 1969) and biological assays with rats. Diets were prepared according to AOAC (1975) except for protein content which was 8% (average = 8.18 ± 0.10, coefficient of variation = 1.22%) instead of 10%. Wistar male rats (7 rats per treatment) weighing 46 to 56g were used. They were maintained individually in metabolic cages with free access to diet and water. The laboratory

Table 1—Proximate composition (d.b.) of wheat, broken rice and defatted soy flours

Composition	Flour		
	Wheat	Broken rice	Defatted soy
Protein (%)	12.35	8.69	50.18
Ether extract (%)	0.91	0.97	1.11
Ash (%)	0.76	0.52	5.75
Crude fiber (%)	0.45	0.73	2.69
Carbohydrate[a] (%)	85.53	89.09	40.27

[a] Calculated by difference.

temperature was 22 ± 2°C with alternating light-dark periods of 12 hr. Protein nutritive value was determined by PER (AOAC, 1975), nitrogen balance, apparent protein digestibility and biological value (Mitchell, 1924), and net protein utilization (Bender and Miller, 1953). Feed efficiency was also calculated according to Dutra de Oliveira and Scatena (1967).

Statistical analysis

The experimental design was a complete block for sensory tests with four samples and an incomplete block for sensory tests with six samples according to Cochran and Cox (1957). Statistical differences between samples were determined by analysis of variance with comparison of means by Tukey's test. The significance for ranked data was analyzed using the Kramer table (Kahan et al., 1973).

RESULTS & DISCUSSION

Characteristics of the flours

The particle size distribution for the three flours was: wheat flour, 80% from 80 to 200 mesh; broken rice flour, 76.8% from 35 to 80 mesh; defatted soybean flour, over 94.0% of the particles were <100 mesh. The proximate composition of the three flours is shown in Table 1. The defatted soy flour had the highest and the broken rice flour the lowest protein content. Wheat and broken rice flours had similar fiber, ash and carbohydrate contents while the defatted soy flour was lowest in carbohydrate and highest in fiber and ash content. The broken rice flour composition was similar to that reported by Bakar and Hin (1985) while the composition of wheat and defatted soy flours was similar to data reported by Yáñez et al. (1982).

Rheological properties of blended flours

The rheological properties of different blended flours and of wheat flour (control) are shown in Table 2. We verified that water absorption capacity increased as defatted soy flour increased (20 to 30%) in the mixture and the broken rice flour decreased (30 to 20%). The wheat flour (control) absorbed 57% water compared with 64.8% for the blended flour with 30% soy and 20% rice. The arrival time was greater for the blended flours than for wheat flour. The values increased as the proportion of defatted soy flour increased. Results were in general agreement with values reported by Gonzalez-Agramon and Serna-Saldivar (1988). The blended flours had increased departure time compared with the wheat flour. The highest values were found for formula II (41 min) and formula III (35 min). All other formulas had departure time of 28 min, compared to 10 min for wheat flour (Table 2).

Time for dough development was also higher for the blended flours (all formulas) than for wheat flour. However, no definite trend could be observed by changing composition of the flour. Various investigators (Gonzalez-Agramon and Serna-Saldivar, 1988; Serna-Saldivar et al., 1988; Yáñez et al., 1982) reported a decrease in time of dough development by mixing soy flour with wheat flour. In our work the highest time for dough development was observed when the defatted soy flour contribution (10–15%) to the mixture was low and the broken rice flour (40–35%) was high. This suggested that rice flour contributed to increased time of dough development.

Dough stability decreased in formulas with high proportions (25–30%) of defatted soy flour and increased in formulas with high proportions (45–35%) of broken rice flour and low proportions (5–15%) of defatted soy flour. Formula IV, with 30% broken rice flour and 20% defatted soy flour, had the same dough stability (9.5 min) as the wheat flour (control). The tolerance index (10 B.U.) was constant for the various formulas and lower than for wheat flour (30 B.U.).

Sensory evaluation

In a preliminary sensory evaluation test, the overall quality of the cookies was considered for the various heat-treatments (180, 210, 240, 270 sec) in the microwave oven. The criterion of selection was overall score above 7.5 on a scale of 1 to 9 points. Based on this criterion, the cookies selected were: formula I 45:5, formula II 40:10, and formula III 35:15% of broken rice and defatted soy flours, respectively, mixed with 50% wheat flour and baked in the microwave oven for 210 and 240 sec (Table 3).

The sensory evaluation scores for flavor and texture of cookies made with formulas I, II and III and baked for 210 and 240 sec in the microwave oven are shown in Table 4. The scores indicated that baking for 240 sec generated the best flavor and texture of all three formulas. Cookies from these three samples were submitted to consumer-type preference test with 75 untrained panelists (Table 5). The scores indicated that formulas I and II composed of 50:45:5 and 50:40:10% of wheat, broken rice and defatted soy flours, respectively, ranked first in preference.

Nutritional evaluation

Proximate composition of cookies produced with the 3 selected formulas appears in Table 6. Protein, ash and fiber contents increased with increasing proportion of defatted soy flour in the formula. Diet efficiency ratio, PER and the main indices of nitrogen utilization are shown in Table 7. The feed efficiency, i.e., diet consumed per unit of body weight gain was significantly better for the formula I diet, worse for the casein diet and intermediate for formulas II and III diets. On the other hand, the PERs of the cookies were inferior to that of casein. Formula I cookies presented the lowest PER. Similar results

Table 2—Rheological properties of blended flours and wheat flour (control)

Formula[a]	Water absorption (%)	Arrival time (min)	Departure time (min)	Dough development (min)	Stability (min)	Tolerance index (B.U.)[b]
I	50.9	7.5	28.5	20.0	21.0	10
II	53.0	10.0	41.0	25.5	31.0	10
III	56.0	17.0	35.0	24.0	18.0	10
IV	60.0	18.5	28.0	21.0	9.5	10
V	62.5	21.0	28.0	22.5	7.0	10
VI	64.8	23.0	28.0	25.5	5.0	10
Control	57.0	0.5	10.0	1.5	9.5	30

[a] See text for components.
[b] Brabender Units.

Table 3—Scores of overall quality of cookies prepared from wheat, broken rice, and defatted soy flours by microwave baking for various times

Formula[a]	Overall quality[f] of cookies microwave-baked for various times (sec) 180	210	240	270	LSD[g]
I	6.93[b]	7.87[a]	7.83[a]	6.40[c]	0.49
II	6.87[b]	7.57[a]	7.67[a]	5.93[c]	0.57
III	6.87[b]	7.53[a]	7.63[a]	5.73[c]	0.51
IV	5.83[c]	6.43[b]	7.10[a]	5.37[c]	0.49
V	5.33[c]	5.77[b]	6.47[a]	4.40[d]	0.31
VI	4.93[b]	5.33[ab]	5.47[a]	4.27[c]	0.48

[a-d] Means followed by different superscripts in a row significantly different at $P \leq 0.05$.
[e] See text for components.
[f] Rating scale (1 = extremely poor; 9 = excellent).
[g] Least significant difference.

Table 4—Sensory evaluation of flavor and texture for cookies prepared from wheat, broken rice, and defatted soy flours by microwave baking

Formula[a]	Baking time (sec)	Flavor[f]	Texture[g]
I	210	7.82[cd]	7.08[b]
I	240	8.04[a]	7.96[a]
II	210	7.80[cd]	7.00[b]
II	240	7.98[ab]	7.88[a]
III	210	7.74[d]	6.96[b]
III	240	7.88[bc]	7.80[a]
LSD[g]	–	0.13	0.12

[a-d] Means followed by different superscripts in a column significantly different at $P \leq 0.05$.
[e] See text for components.
[f] Rating scale (1 = extremely poor; 9 = excellent).
[g] Least significant difference.

Table 5—Scores of the rank order of consumer-type preference test of cookies

Formula[a]	Baking time (sec)	Scores of ranked order 1	2	3	Total[b]
I	240	29	60	48	137
II	240	31	54	51	136
III	240	15	36	126	177
Sum of total					450

[a] see text for components
[b] Rank totals required for significance at $p < 0.05$ for 3 treatments and 75 panelists are 135–165; therefore, formula III (less preferred) was significantly different from formulas I and II (more preferred).

Table 6—Proximate composition (moisture basis) of preferred cookies

Formula[d]	Composition (%) Moisture	Protein	Ether extract	Ash	Crude fiber	Carbohydrate[e]
I	2.98[a]	7.82[c]	12.08[a]	0.93[c]	0.43[a]	75.76[a]
II	3.00[a]	9.16[b]	12.12[a]	1.50[b]	0.49[a]	73.73[b]
III	3.06[a]	10.41[a]	12.19[a]	1.73[a]	0.56[a]	72.05[c]
LSD[f]	0.39	0.49	0.21	0.13	0.14	1.07

[a-c] Means followed by different superscript in a column are significantly different at $P \leq 0.05$.
[d] See text for components.
[e] Calculated by difference.
[f] Least significant difference.

were reported by Gonzalez-Agramon and Serna-Saldivar (1988): PER values of 2.10 and 2.11 for tortillas fortified, respectively, with 11.1% defatted soy flour or 5.6% isolated soy protein, which were inferior to 2.77 for casein, but superior to 1.06 for tortillas made with wheat flour. Suárez (1958) reported an increase of 9.1% in the PER of bread made with 70% wheat and 30% rice, compared with 100% wheat bread. Results indicated that replacement of wheat flour by rice and defatted soy flour improved the PER value of the mixture.

There was no difference in nitrogen retention (Table 7) in replacement of wheat flour by broken rice and defatted soy flours in the mixtures. Apparent protein digestibility, biological value and NPU_a were superior for the casein diet over

Table 7—Nutritional quality of cookies prepared from blends of wheat, broken rice and defatted soy flours by microwave baking

Formula[d]	FE[f]	PER[g]	NB[h] (g)	D_a[i] (%)	BV_a[j] (%)	NPU_a[k] (%)
I	7.03[a]	1.75[c]	0.58[a]	92.31[c]	85.41[b]	78.84[b]
II	6.04[a]	2.06[b]	0.58[a]	92.98[bc]	85.18[b]	79.22[b]
III	5.48[a]	2.26[b]	0.67[a]	93.77[b]	88.92[b]	83.35[b]
Casein[e]	4.53[b]	2.67[a]	0.68[a]	95.89[a]	94.29[a]	90.41[a]
LSD[l]	0.78	0.31	0.12	1.21	4.88	4.77

[a-c] Means followed by different superscripts in a column significantly different at $P \leq 0.05$.
[d] See text for components.
[e] Commercial casein with 84.6% purity.
[f] FE–feed efficiency.
[g] PER–protein efficiency ratio.
[h] NB–nitrogen balance, nitrogen retained by 7 rats during 5 days.
[i] D_a–apparent protein digestibility.
[j] BV_a–apparent biolog cal value.
[k] NPU_a–net protein utilization.
[l] Least significant difference.

formulas I, II and III blends (Table 7). However, no statistically significant differences were found between the three formulas. According to the Protein Advisory Groups of the United Nations System (PAG, 1971), the NPU_a value for milk and cereal based product should be 60 minimum. Therefore, the formula cookies seemed to be adequate in terms of protein content and utilization.

Our results support the conclusion that a blended flour with 50% wheat, 40% broken rice and 10% defatted soy flours or 35% broken rice and 15% defatted soy flours, baked in a microwave oven for 240 sec, could be used to produce cookies with good sensory properties, acceptability and adequate nutritive quality. Tsen et al. (1977) demonstrated that baking bread with microwaves improved protein nutritive value when compared with conventional baking.

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Starch Gelatinization in Chemically Leavened Bread Baking

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ABSTRACT

Differential scanning calorimetry was employed to assess the kinetics of the starch gelatinization in bread doughs. The process of gelatinization, which depended on the depth from the surface of the sample, followed the same first order kinetics in the baking treatment and in the calorimetric scan. Its progress was predictable when the local temperature could be suitably recorded. Accordingly, thorough temperature control allowed monitoring starch gelatinization and bread baking.

Key Words: starch, gelatinization, leavening, bread, calorimetry

INTRODUCTION

GELATINIZATION can be referred to as the disaggregation of starch granules within an aqueous environment at suitable temperature. This change takes place in many processes in food technology, where, as in baking, it is relevant to the final quality of the product. The gelatinization change is simple and peculiar. At a given temperature within the 50–70°C range, depending on the kind of starch and its origin, the starch granules undergo a progressive swelling due to hydration. Their boundaries appear less clearly defined, while the typical birefringency vanishes and a more or less homogeneous gel is eventually formed.

In spite of the large number of studies directed toward a better insight of the process, only in the last decade have X-ray diffraction and differential scanning calorimetry (DSC) allowed development of reliable models for starch structure and its gelatinization (Biliaderis et al., 1980, 1986). According to one hypothesis, starch is a dispersion of amylose and amylopectin crystals within a non-crystalline matrix. This matrix in turn is formed by amorphous polysaccharides and semicrystalline material. X-ray diffraction has allowed the recognition of different crystal structures, according to the origin of the starch and its previous thermal history. These structures have been designated as A, B, C, and V forms (Zobel, 1988). Previous thermal treatment and the presence of either water or alcohols and lipids would allow transitions among those crystal types (Zobel, 1988).

In the temperature range where gelatinization occurs, DSC traces show a broad peak which has generally been interpreted as a sign of simultaneous changes (i.e., accounting for the sum of a large number of small contributions), such as swelling of the amorphous regions of the starch granules and melting of crystalline areas (Biliaderis et al., 1980, 1986). Such melting behavior would depend on hydration and swelling of the former. When hydration is large, i.e., 80–90%, swelling would destabilize the crystalline region which undergoes melting at about 60°C. In such cases, the DSC trace shows a single well defined endothermic peak. When hydration remains below 70%, the fusion is still in progress at 70°C and is exhausted only at higher temperature (about 100°C). In those cases, the DSC trace can show a double peak. More extreme conditions of hydration are again characterized by a single endothermic peak, which is, however, shifted beyond 100°C.

Regarding kinetics of starch gelatinization, the studies of simple water/starch systems (Kubota et al., 1979; Lund and Wirakartakusumah, 1984) support tentatively a first order kinetics, i.e.,

$$(1 - a) = \exp(-Kt)$$

where a is the gelatinized fraction, K is the kinetic constant and t the time after onset of the process.

Some kinetic results relating to gelatinization in baking have been reported (Donovan, 1977; Mizukoshi et al., 1979; Wootton and Bamunuarachchi, 1980; Ghiasi et al., 1982; Olsson and Skjöldebrand, 1983; Horton et al., 1990; White and Lauer, 1990). The objective of our work was to assess the kinetics of starch gelatinization during chemically leavened bread baking by means of differential scanning calorimetry.

MATERIALS & METHODS

Bread preparation and baking

Samples of leavened bread were obtained from doughs [in the proportion: flour (100 g), aqueous solution of baking powder (58.5g water and 6.0g baking powder), NaCl (1.5 g)] which were stirred for 5 min in a Hobart N50 G kneading machine (speed 1). pH of the aqueous solution of the baking powder was 6.83. Dough samples (about 205g) were placed in Pyrex glass cylinders (9.2 cm diam, 10 cm height) and, after 15 min rest at room temperature (about 20°C), baked at 205°C for 28 min in an electric oven (Moretti model Mikro - Italy). To minimize the heat supplied by radiation, the incandescent heating elements on the back wall of the oven were screened by a cement panel. A glass holder was used to support the sample over the bottom to reduce the heat supply by conduction. The heat transfer to the sample could thus be considered to be due principally to convection. Air flow across the specimen was about 1.4 m/sec. The simple cylindric shape of these specimens (4.6 cm radius, 5.7 cm height) before baking allowed quick and reproducible sample preparation.

Calorimetric determinations

Differential calorimetry determinations were carried out on samples of both fresh and partially baked doughs corresponding to different baking times, i.e., 3, 5, 7, 10, 12, 14, 16 and 18 min. Loaves were sliced to obtain specimens of the central core, under-crust (1.3 cm depth) and crust.

The instruments employed were a Mettler DSC 20 (Mettler Instrument AG, Switzerland) and a differential flux calorimeter Setaram C80 (Setaram, France). These differed in the kind of cells used (sealed aluminum pans and screwed stainless steel cylinders, respectively), sensitivity (5 and 2 J/g), scanning rates (5 and 0.5°C/min) and sample mass (40 and 1000 mg). An empty cell and an Al_2O_3 loaded cell were employed as references in the Mettler and Setaram calorimeter, respectively. The Al_2O_3 load (about 2.6 g) was defined to counterbalance the overall heat capacity of the sample under study. The Mettler calorimeter was calibrated by checking temperature and enthalpy of fusion of indium, lead and zinc. The calibration of the Setaram calorimeter was carried out by means of a special device (Setaram power unit EJ2) capable of producing a given Joule effect. A further calibration was carried out by determination of the heat capacity of pure water throughout the temperature range adopted.

Recordings were obtained over the 30–110°C range with both calorimeters. For evaluation of the thermal effect due to gelatinization, the two instruments appeared to produce equivalent results. Therefore, most results were obtained with the Mettler DSC 20 which was simpler and faster. On the other hand, recordings with the SETARAM C80 calorimeter (which required more than 3 hr for the same temperature scan) had no notable "noise" and were, therefore, suitable

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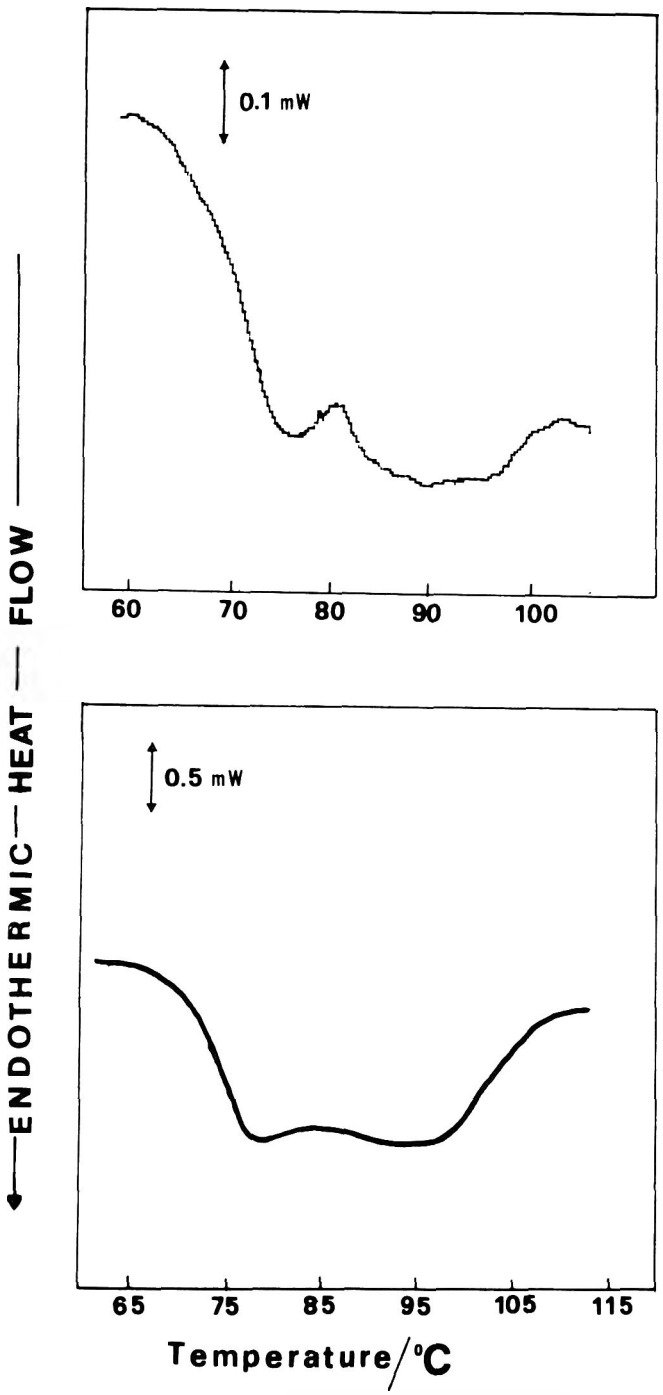

Fig. 1—Calorimetric traces of fresh dough samples obtained with the Mettler DSC 20 (upper) and SETARAM C80 (lower) calorimeters.

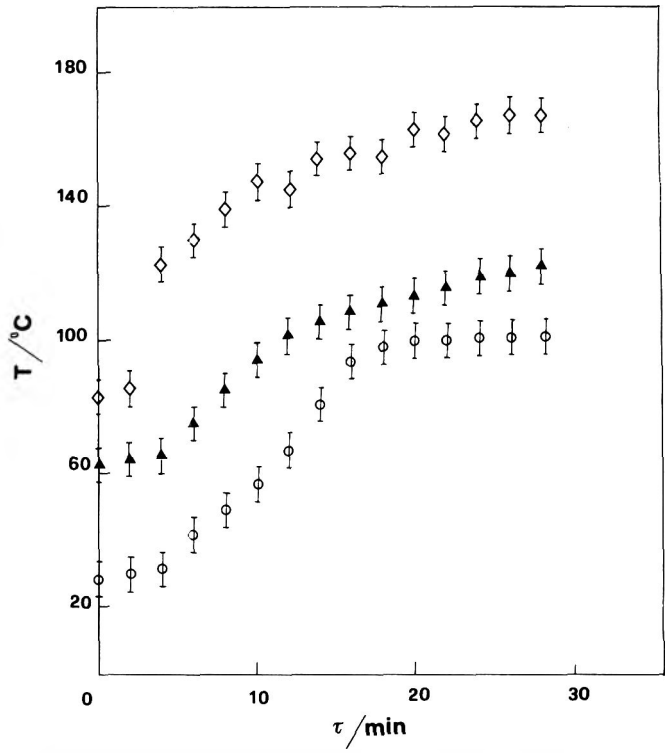

Fig. 2—Temperature (T) vs baking time (τ) for crust (◇), under-crust (▲) and core (○). Bars correspond to confidence limits.

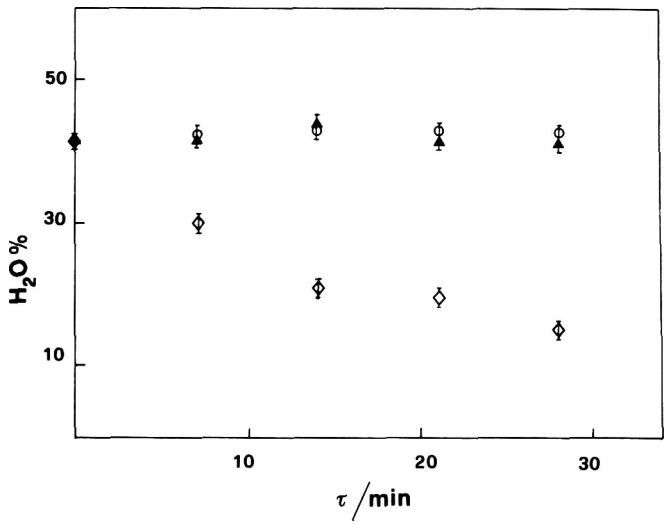

Fig. 3—% H_2O vs baking time (τ) for crust (◇), under-crust (▲) and core (○). Bars correspond to confidence limits.

for direct analysis of the peak profile, which was stored in an on-line data processor.

Comparison between two samples of the same fresh dough investigated with the two calorimeters is shown in Fig. 1. In both cases the peculiar double peak appeared and its area accounted for 3.4 ± 0.3 J/g.

Determination of temperature and moisture

The temperature of the core, under-crust and crust during baking was measured in triplicate by five copper-constantan thermocouples (2 mm diam, 0.1°C sensitivity) connected to an ATS-6 amplifier/transmitter provided with a ATI-2 display (Ing. C. Pavone, Italy). The moisture of the samples was determined during baking (0, 7, 14, 21, 28 min) as follows: they were sliced, quenched in liquid nitrogen (to minimize water loss) and stored frozen at −20°C. Each moisture value was the average of three results, obtained after drying in an oven at 105°C to constant weight.

RESULTS & DISCUSSION

Gelatinization degree

The process of starch gelatinization during baking appeared to depend on the depth from the surface of the sample, probably due to differences in heat and mass transfer. The greater the depth from the surface, the slower the baking rate. The temperature increase at the surface (crust) was much faster than at medium-depth (under-crust) and in the core region (Fig. 2), while moisture showed a substantial decrease only in the crust (Fig. 3). Experimental evidence (see below) suggested that both parameters would directly affect the gelatinization process, which did not start until a minimum critical temperature was

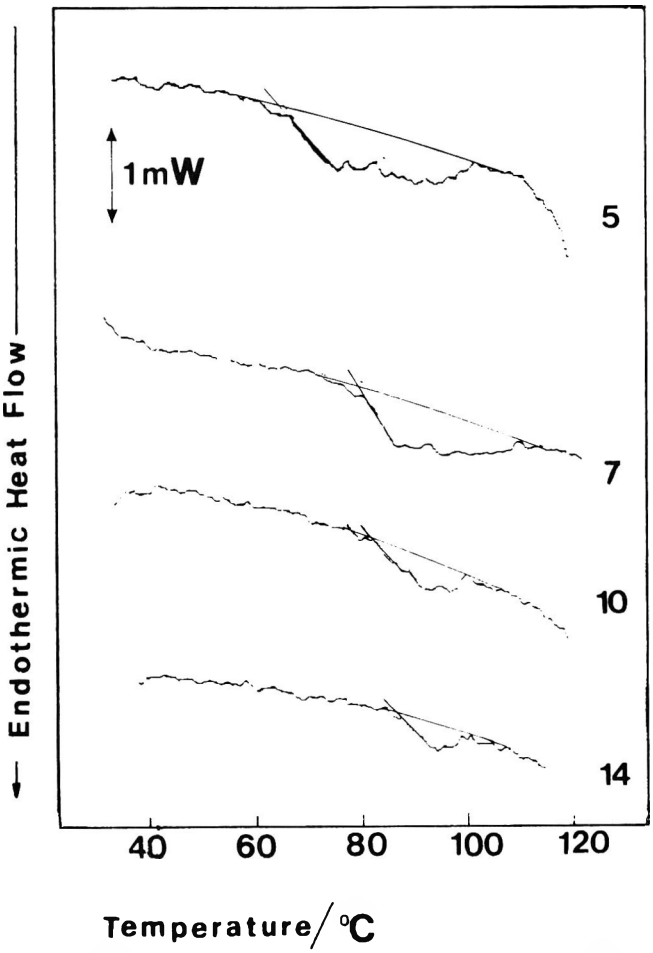

Fig. 4—Differential calorimetry traces of partially baked undercrust samples (at different baking times 5, 7, 10, 14 min). The point of intersection with the base line represents the initial gelatinization temperature (Ti).

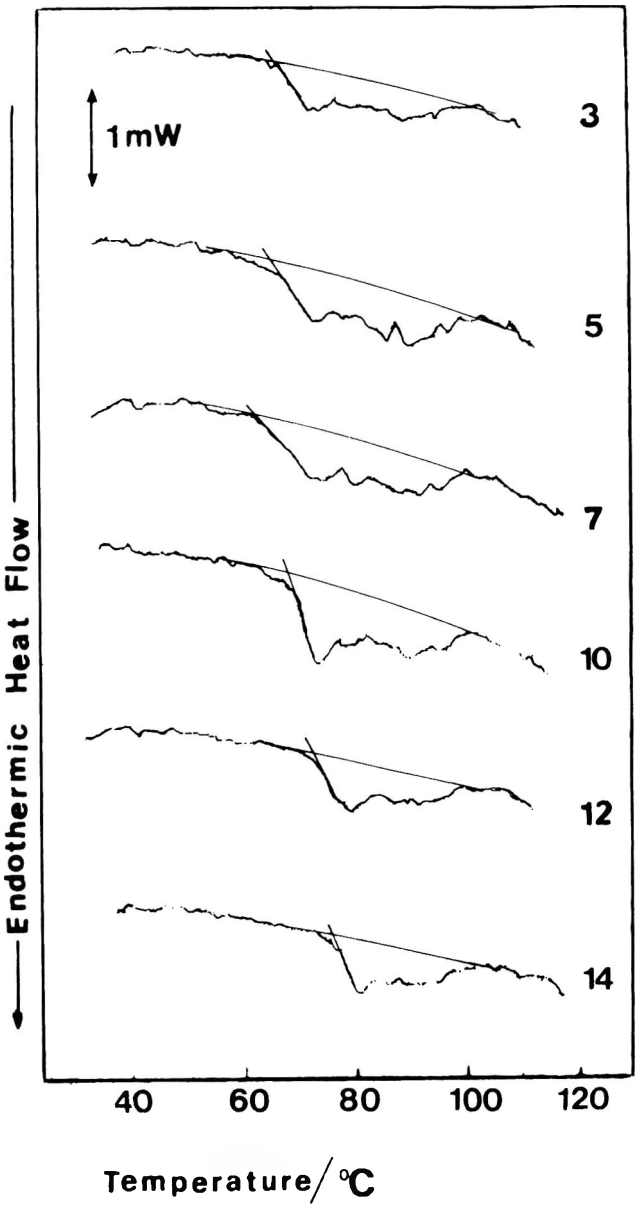

Fig. 5—Differential calorimetry traces of partially baked core samples (at different baking times 3, 5, 7, 10, 12, 14 min). The point of intersection with the base line represents the initial gelatinization temperature (Ti).

reached and could progress only in the presence of adequate moisture.

Dough specimens were characterized by their calorimetric trace where the endothermic peak of gelatinization occurred. The area of this peak corresponded to the heat of gelatinization as follows: when the moisture content was adequate to fully gelatinize the starch, the peak area was maximum. Doughs which had previously undergone partial baking in the oven showed traces with smaller gelatinization peaks, since part of their starch content had already gelatinized. The DSC investigation, therefore, revealed the amount of gelatinization which took place during the scan (Fig. 4 and 5). Fully baked samples showed flat calorimetric traces (not shown in the figures).

Crust, under-crust and core samples removed after different baking times, were examined. After 5 min, no peak appeared in the trace from crust samples, while the traces obtained from undercrust and core samples were like those reported in Figs. 4 and 5, respectively. Table 1 summarizes the experimental data.

Samples of less gelatinized doughs gave traces with a double endothermic peak. This was in agreement with the results reported for starchy systems with analogous moisture content (Biliaderis et al., 1980) and interpreted as two-phase processes. The relevant gelatinization peak areas decreased with increasing baking time along with a progressive shift of the peak onset toward higher temperature. This might be explained by considering that the process of baking implies the propagation of a gelatinization front and a decrease in available water. The split of the peak became progressively less evident and, for the under-crust sample baked for 14 min, it changed to a single - peak signal at high temperature. This also was in agreement with observation reported (Donovan, 1977) for drier starchy products.

Table 1—Relation between initial gelatinization temperature (Ti), gelatinization degree (α), and baking times (τ)

	Crust		Under-crust		Core	
τ (Min)	Ti (°C)	α	Ti (°C)	α	Ti (°C)	α
0.00	60.00	0.00	60.00	0.00	60.00	0.00
3.00	61.00	0.50	n.e.[a]	n.e.[a]	60.50	0.00
5.00	–	1.00	60.00	0.16	61.40	0.00
7.00	–	–	71.00	0.24	59.50	0.00
10.00	–	–	76.80	0.31	63.30	0.06
12.00	–	–	n.e.	n.e.	71.40	0.15
14.00	–	–	85.00	0.72	74.00	0.36
16.00	–	–	–	1.00	80.00	0.61
18.00	–	–	–	–	–	1.00

[a] n.e. = not evaluated.

The shift of peak onset, i.e., the start of gelatinization process,

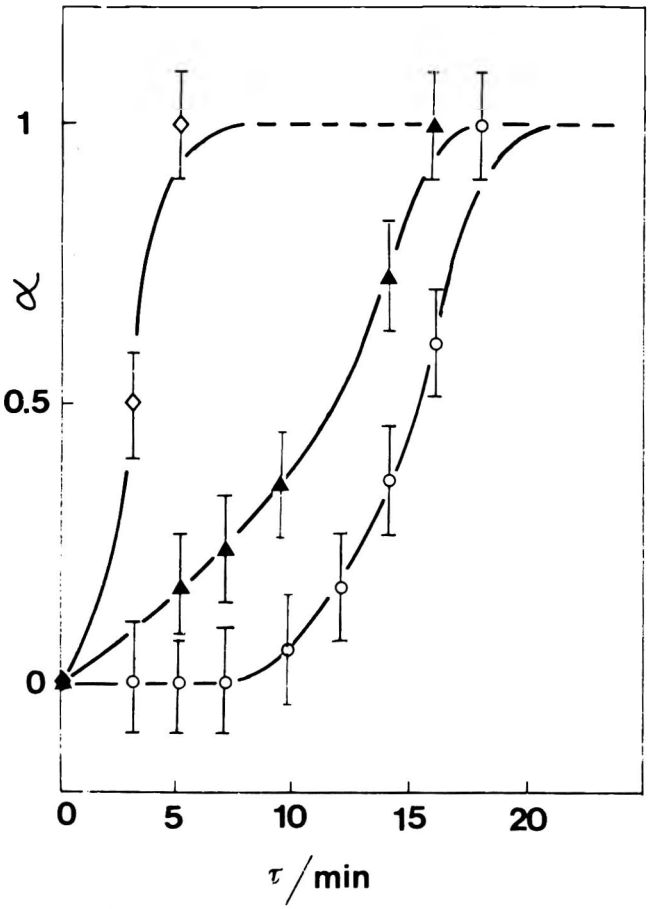

Fig. 6—Gelatinization degree (α) vs baking time (τ) for crust (◇), under-crust (▲) and core (○).

may be described as follows. The temperature of the initial gelatinization (Ti), close to 60°C for better hydrated samples (i.e., fresh doughs), showed a shift for partially baked samples with a two-phase trend. In the early phase of baking, 7 min for the core and 5 min for the undercrust, it remained close to 60°C. After that phase it increased with baking time, τ, according to:

$$Ti/°C = 42 + 3.9 \times 10^{-2}\ \tau/sec \quad (1)$$

$$Ti/°C = 51 + 4.2 \times 10^{-2}\ \tau/sec \quad (2)$$

for the core and the under-crust, respectively.

The only significant difference, therefore, dealt with the length of constant-Ti period, which was shorter for the under-crust than for the core. The calorimetric data were treated in terms of gelatinization degree defined as:

$$\alpha(\tau) = 1 - Q(\tau)/Qmax \quad (3)$$

where Q(τ) and Qmax were the thermal effects evaluated for the calorimetric scan of partially baked and fresh dough samples, respectively. They were related to the residual starch gelatinization after τ and zero baking times, respectively, in a given region of the dough.

The experimental $\alpha = \alpha(\tau)$ trends were different for the various dough regions (Fig. 6). The crust underwent a quicker gelatinization than the deeper regions, since the faster the heat transfer, the shorter the time to attain T = Ti. In spite of the fact that the time required for complete baking was 28 min, starch gelatinization appeared complete in all dough regions after only 18 min.

Kinetics of starch gelatinization

The trend of α vs time and temperature can be drawn from a single scan of a fresh dough sample in the Setaram C80 calorimeter by mathematical analysis of the peak profile (Fig. 7). It was assumed, as a first approximation and as reported by others (Lund and Wirakartakusumah, 1984), that starch gelatinization would follow first order kinetics, i.e.,

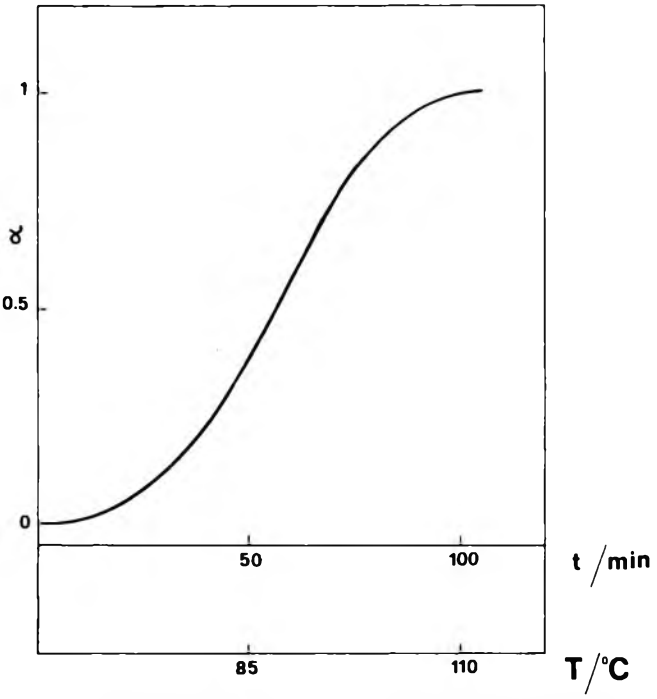

Fig. 7—Gelatinization degree (α) of a fresh dough sample vs scanning time (t) and temperature (T) in the SETARAM C80 calorimeter. The plot directly corresponds to the integration of the trace peak during the scan. The results are obtained from the on line processor.

$$(1 - \alpha) = \exp(-Kt) \quad (4)$$

where t is the time elapsed from the starch of the gelatinization process, i.e.,

$$t = \tau - t' \quad (5)$$

t' being the time required to attain the temperature of initial gelatinization Ti [see above Eq. (1) and (2)].

According to the Arrhenian behavior of K, the following expression could be drawn:

$$\log(1 - \alpha) = -K^{\circ}(\tau - t')\exp(-Ea/RT) \quad (6)$$

K° and Ea being the preexponential term and the activation energy, respectively.

For practical use, Eq. (6) was rewritten in a log form, i.e.,

$$F(T) = \log(-\log(1-\alpha)/(\tau - t')) = \log K^{\circ} - Ea/RT \quad (7)$$

which is a straight line in the plot F(T) vs 1/T.

The fit of the experimental data gave:

$$K^{\circ} = 6.21 \times 10^{9}\ sec^{-1} \text{ and } Ea = 93\ KJ\ mol^{-1}$$

in agreement with published reports (Lund and Wirakartakusumah, 1984; Zobel, 1988).

We verified that the above kinetic model would also apply to gelatinization during baking, as shown by the data obtained from a series of partially baked doughs (Table 1). The results for the core are shown in Fig. 8, where the experimental data are compared with the trend reported in Fig. 7. The analogous plot for under-crust and crust samples appeared much more scattered due to the larger error range (not reported).

These trends could be considered satisfactory since the kinetics of gelatinization in the oven are obtained from data relevant to various samples, corresponding to different gela-

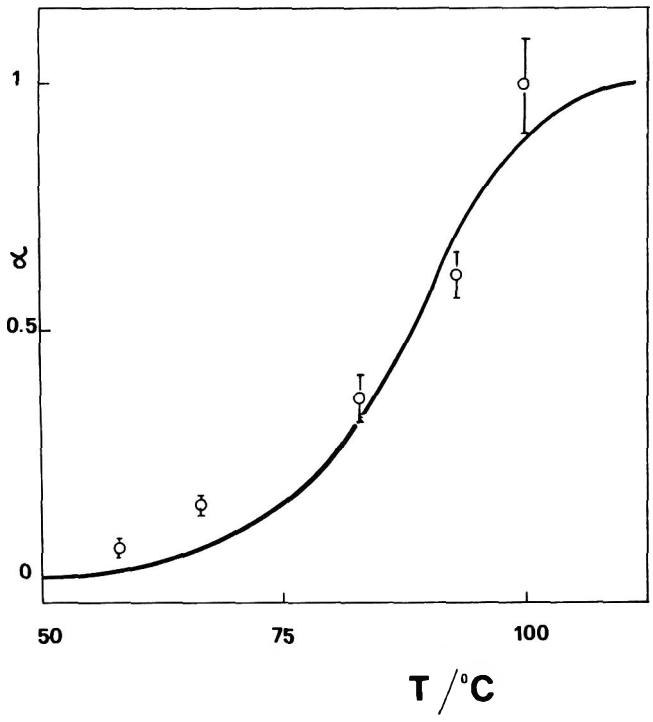

Fig. 8—Gelatinization degree (α) vs temperature (T). Continuous line corresponds to the plot of Fig. 7, whereas data points are obtained from the calorimetric analysis of five partially baked samples.

tinization fractions, whereas that in the calorimeter derives from one sample undergoing the entire process. This would support the conclusion that, at least for the early steps of the process ($\tau \leq 18$ min), baking and gelatinization correlated with each other, i.e., the progress of the one could be assessed by defining the increase of the other. These results showed a direct correlation between gelatinization degree (α) and temperature (T) (for $T > Ti$), both in the oven and in the calorimeter (Fig. 8).

Kinetic analysis (Fig. 8) has thus shown that, once started, the starch gelatinization would progress in a way that would be predictable, if the local temperature were suitably recorded. Temperature monitoring would, therefore, allow a reliable control of starch gelatinization and the corresponding baking progress. Further investigations have been recently undertaken to verify the possibility of analogous conclusions for more complex starchy systems where extra-ingredients would significantly affect starch gelatinization.

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Monitoring Freeze-Drying by Low Resolution Pulse NMR: Determination of Sublimation Endpoint

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ABSTRACT

An NMR method was developed for detection of the sublimation period endpoint during the freeze-drying of potato pieces. Longitudinal (T_1) and transverse (T_2) relaxation times were estimated using rapid NMR methods. Samples were immediately examined to determine presence of a frozen core. The plot of T_1 vs T_2 showed an abrupt increase at a point corresponding to the complete elimination of the frozen core. Similar T_1 vs T_2 curves were observed when monitoring freeze-drying of a single potato piece inside the NMR probe-head. The detection of an anomalous freeze-drying is possible by monitoring the T_1, the T_2 or the free induction decay signal.

INTRODUCTION

FREEZE-DRYING is a low temperature drying process where most of the water is eliminated by sublimation from a thin front on the surface of a frozen core which gradually recedes into the product. This process leads to high quality products (color, shape, aroma, rehydration). However, the development of freeze-dried foodstuffs entails relatively high production costs. The sublimation of the water requires a reduction of the temperature and partial vapor pressure in the product to values below those of the triple point of water (0.01°C and 610.7 Pa for pure water), and a heat supply for the change of phase (2800 J/g of ice at 0°C).

At present, the piloting of industrial freeze-drying is largely empirical and this incurs very wide security margins. Any improvement in productivity of freeze-drying would require better knowledge of the process. Modeling of the dehydration kinetics can be used to predict advancement of the process as a function of operative parameters (King, 1971; Wolff et al., 1989). However, simple practical modeling methods which could be used under industrial conditions, are based on sublimation-related phenomena and do not take into account other water desorption processes (Wolff et al., 1989). As a consequence, such models cannot accurately predict the sublimation phase endpoint. However, at that point the pressure and temperature settings could be optimized to reduce energy consumption and equipment operation time.

One possibility for detecting the sublimation phase endpoint would be by means of indirect measurements, such as the partial pressure of water (Bouldoires, 1969). Such methods are however still in the experimental stage. Another approach is to sample and analyze the product during the freeze-drying process. Freeze-drying equipment manufacturers are at present developing such sampling devices. Thus, the development of rapid analytical methods which provide direct information related to the binding strength and mobility of water molecules is of great importance. Nuclear magnetic resonance (NMR) is one of the methods that has been used to determine the state of water in foods (Berendsen, 1975; Beall, 1983; Labuza, 1985).

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Detailed NMR studies of water mobility require the use of ^{17}O as the probe nucleus in order to avoid the chemical exchange and cross-relaxation effects encountered with proton NMR (Richardson and Steinberg, 1987). However, with industrial drying it is impossible to use ^{17}O, its natural abundance being so low, especially at lower water contents (Richardson et al., 1987). Proton low resolution pulse NMR, although influenced by factors related to nonwater constituents, provides valuable information on the mobility of the water molecules. In fact, its response depends on the different proton populations present, their size and relaxation characteristics — which in turn depend on their mobility. The mathematical treatment of the proton NMR signals of food products can serve to define different water fractions and has been used in the study of samples at various water contents (Leung et al., 1976, 1979; Brosio et al., 1983, 1984; Ratkovic, 1987).

The freezing behavior of water reflects the general physicochemical state of the water in the product (Simatos and Karel, 1988). Nagashima and Suzuki (1985) developed a computer method to study the state of water, based on the fact that protons in the liquid and solid phases can be distinguished by pulse NMR, and that the "bound water" does not freeze below 0°C. This definition of "bound water" is widely used, as is the application of pulse NMR techniques to quantification of liquid protons in sub-zero temperature samples (Toledo et al., 1968; Leung and Steinberg, 1979; Weisser and Harz, 1984). At sub-zero temperatures the "free water" is frozen, and practically all the macromolecular protons are immobilized, so that only part of the water of hydration is sufficiently mobile to give an NMR signal (Nagashima and Suzuki, 1985).

Our objective was to develop a pragmatic approach for monitoring the hydration state during freeze-drying of potato samples. The specific objective was to define a rapid NMR method correlated with the presence of ice, in order to determine the sublimation period end point.

MATERIALS & METHODS

Sample preparation

Potatoes were prepared by stamping out cylinders (diam = 7 mm; h = 3 cm). Size was chosen to fit 10 mm outside diameter NMR tubes, to have the greatest possible signal intensity and the smallest possible random errors. The cylinders were cooled to −20°C for at least one day, and freeze-dried in a single layer using the traditional vacuum freeze-drying contact process on a USIFROID SMH15 model machine (Maurepas, France), previously described by Wolff et al. (1989). Identical experiments were carried out over different times, with plate temperature 50°C and condensing plate temperature −65°C, working pressure 50 Pa measured by capacitive sensor. At the end of the drying time, care was taken to rapidly transfer the cylinders to pre-cooled NMR tubes and to store them at −20°C to avoid melting of the frozen core or diffusion of water. After storage for 2 days at −20°C, samples were stabilized in a water bath at −7°C for 15 to 18 hr, before being analyzed by NMR, examined for presence of a frozen core and water content determined. Three replicates of this experiment were carried out (results in Figs.2, 3 and 4 correspond to one).

For the study of the external dry layer, potatoes were cut into parallelepipeds (1 cm^2 × 3 cm) and freeze-dried over different times as described. After freeze-drying, the samples were cooled to -20°C and the external dry layer was carefully separated from the frozen core using a cutter and then transferred to pre-cooled NMR tubes. Five

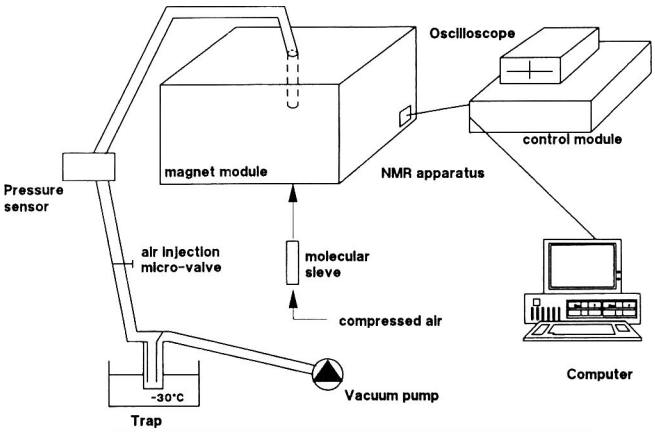

Fig. 1—Experimental setup for freeze-drying in the NMR apparatus.

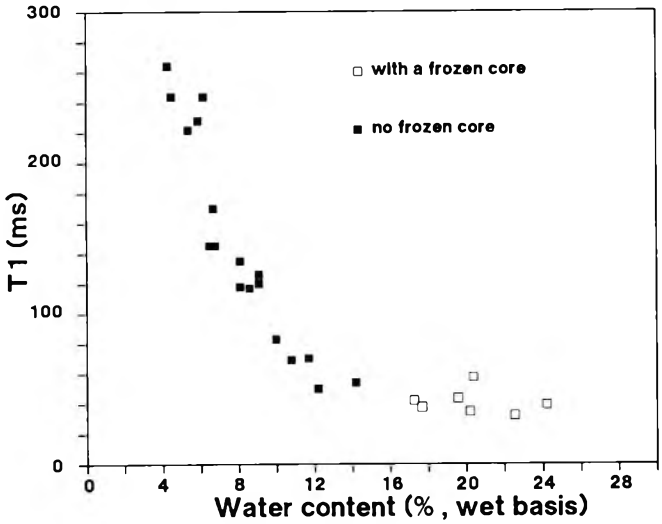

Fig. 2—Evolution of T_1, and disappearance of the frozen core, as a function of water content during the freeze-drying of potato cylinders in a pilot plant.

parallelepipeds were used to prepare each sample for a given freeze-drying time. After storage for 6 days at −20°C, the samples were stabilized in a water bath at −7°C for 15 to 18 hr, before being analyzed by NMR and the water content determined.

NMR measurements

A low resolution pulse NMR apparatus was used (Minispec pc120 Bruker, Rheinstetten, West Germany). To avoid condensation inside the sample probe and on the outer walls of NMR tubes, a continuous flow of dried air was used. The temperature in the sample probe was maintained at −7°C. An audio filter bandwidth of 1 MHz, a diode detector, a tenfold signal to noise ratio enhancement (ENH) and a 1.5 sec relaxation delay (RD) between repetitions of the pulse sequence were used. An ENH = 6 and an RD = 1 sec were preferred for monitoring freeze-drying experiments carried out inside the NMR apparatus sample probe. In such cases the sample is not in equilibrium and measurements must be done as quickly as possible.

The amplitudes of the signal 11 μsec after a 90°-*t*-90° pulse sequence, used for estimation of longitudinal relaxation time (T_1), was designated as M_t. The amplitude of the free induction decay (FID) signal x μsec after the first 90° pulse was FID_x. T_1 estimates were calculated using FID_{11} and M_t values, for t equal 7 and 80 msec, as the quantity $\log(FID_{11} - M_t)$ is a linear function of t with slope $-1/T_1$. Transverse relaxation time (T_2) estimates were calculated from FID_{11} and FID_{22} as the logarithm of FID_x is a linear function of x with slope $-1/T_2$ (Farrar and Becker, 1971). Two separate pulse sequences were used to obtain FID_{11} and M_7, and FID_{22} and M_{80}.

For the above values of x, the T_2 estimate depends mainly on the fast relaxing protons, whose transverse relaxation is usually best described by a gaussian model. However, for carrots and potatoes with water contents above 10%, the FID curves between 11 and 25 μsec are best fitted using an exponential model (Monteiro Marques *et al.*, 1991), probably because of magnetic communication between the different proton populations present (Bryant and Shirley, 1980).

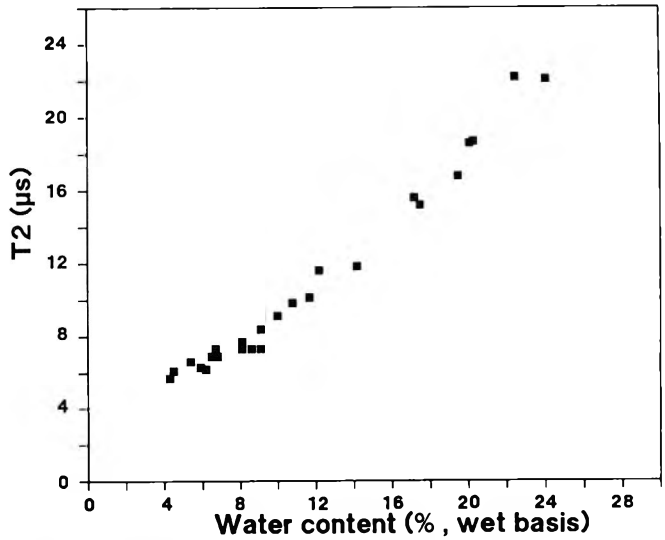

Fig. 3—Evolution of T_2 as a function of water content during the freeze-drying of potato cylinders in a pilot plant.

Note that what we designated T_1 and T_2 are in fact empirical parameters related to weighted averages of distributions of T_1 and T_2 for the different proton populations. However, the NMR methods we used have the advantage of not requiring determination of sample weight and are very rapid. Total time for acquisition of data to calculate both T_1 and T_2 was < 80 sec when ENH = 6 and RD = 1 sec.

Detection of frozen cores

After NMR measurements, the cylinders were cut open and both sections were rapidly examined. When a frozen core was present, there was a clear demarcation between it and the external dry layer. The water content was determined by weight loss, after drying for 20 hr at 105°C, and expressed in kg water/100 kg freeze-dried product. After performing NMR measurements, the tared NMR tube was opened, weighed on an analytical balance and placed in the oven.

Freeze-drying in the NMR apparatus

To monitor the freeze-drying in real time, the process was carried out inside the probe-head, as described in Fig. 1. A parallelepiped of potato was frozen at −20°C and placed in an NMR tube connected to a vacuum pump with an upstream trap at −30°C. The pressure was regulated at 100 Pa by air injection through a micro-valve and measured by a capacitive sensor. When the operative pressure was reached, the tube was introduced into the NMR apparatus probe-head, thermostated at 25°C. For NMR monitoring of freeze-drying we used a program (EXP ___ SUP, Bruker) to define measurement conditions for determining T_1 and T_2, to set the interval between relaxation time determinations, and to manage data.

Parallelepipeds were preferred to cylinders for *in situ* freeze-drying, because fusion invariably occurred in the cylinders. Fusion also occurred in 3 of the 6 experiments with parallelepipeds.

RESULTS & DISCUSSION

Development of a rapid method for monitoring the freeze-drying process

Cylindrical potato samples were dried in a freeze-drying pilot plant and analyzed by low resolution NMR just before visual examination for the presence of a frozen core and water content determination. As shown in Fig. 2, T_1 increased by a factor 4 for water contents between 12 and 5%. T_1 could be of use for rapid determination of water content. In fact, the

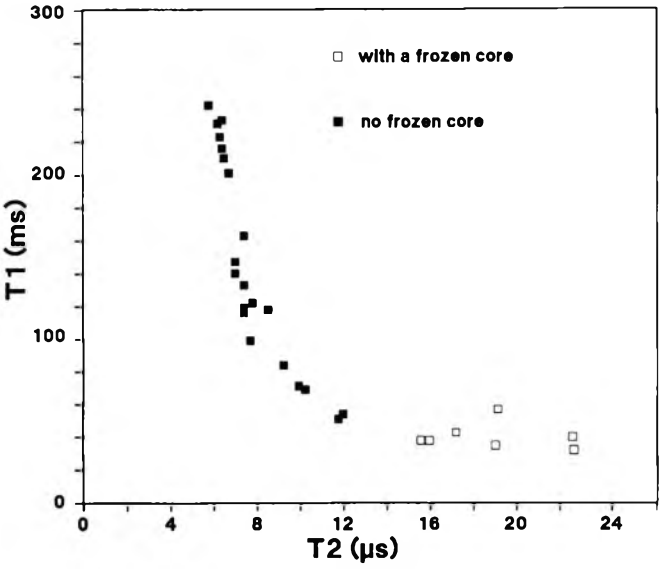

Fig. 4—Characteristic NMR curve for monitoring the freeze-drying of potato cylinders in a pilot plant.

precision of the method was probably better than it appears from Fig. 2, because of the errors due to manipulation that preceded the water content determination. During an industrial freeze-drying process many samples must be analyzed to monitor the process and to take into account the distribution of water contents of the product. It would thus be possible to follow the average water content during the last phase of drying.

The abrupt increase in T_1 as the water content dropped below 14% approximately corresponded to the complete elimination of the frozen core, that is, to the sublimation period end point (see Fig. 2). At that point the drying process could be continued at atmospheric pressure without deforming the product and with obvious economic advantages: lower energy consumption and less time in the freeze-drying chamber.

The localization of a particular point on a curve obviously requires two values. Unfortunately, in this case the drying time could not be used because small differences in samples lead to different water contents for a given drying time. The water content also could not be used as its determination for each sample would complicate automation of the system and increase the response delay. As can be seen in Fig. 3, the T_2 was almost proportional to water content. It could thus be used as the second value to follow the evolution of T_1 (Fig. 4). The T_2 has the additional advantage that it can be calculated from data obtained during the same pulse sequence used for determination of T_1. Consequently, by using a rapid method of determining both T_1 and T_2 it was possible to follow a freeze-drying experiment. Moreover, this method could be used to determine a point of technological interest, the end of the sublimation period.

Monitoring freeze-drying in the NMR probe-head

Figure 5 shows the evolution of T_1 as a function of T_2 observed during two freeze-drying experiments. Curve A is similar to Fig. 4 whereas curve B is completely different. The examination of the samples after freeze-drying showed that curve A corresponded to successful freeze-drying while curve B corresponded to a sample which had undergone fusion and become deformed. As a consequence of the greater water mobilities that resulted from the fusion of ice the T_1, the T_2 and the FID signal all increased. The fusion was probably complete when curve B attained the T_1 maximum. From that point on, the curve was similar to those observed for samples dehydrated by other methods (Zimmerman and Lasater, 1958; Leung et al., 1976; Nakano and Yasui, 1979; Ratkovic, 1987; Monteiro Marques et al., 1989). These present a T_1 minimum as explained by the theory of Bloembergen, Purcell and Pound (Bloembergen et al., 1948).

The detection of an anomalous freeze-drying would be possible by monitoring either the T_1, the T_2 or directly from the FID signal. In fact, the observation of the FID signal on an oscilloscope was quite informative. If fusion occurred the signal intensity increased, instead of decreasing as shown in Fig. 6. This presents the evolution of the FID during a normal freeze-drying process. The decrease in signal intensity of slow relaxing protons, as drying proceeds, may be due to existence of magnetic communication between mobile water protons and solid protons (Bryant and Shirley, 1980), the latter being gradually removed by sublimation. It could also be from the desorption of unfreezable water, or of relatively slow relaxing protons, from the "dry layer" in the periphery of the sample. Figure 7 and the results presented in Table 1 agree with the

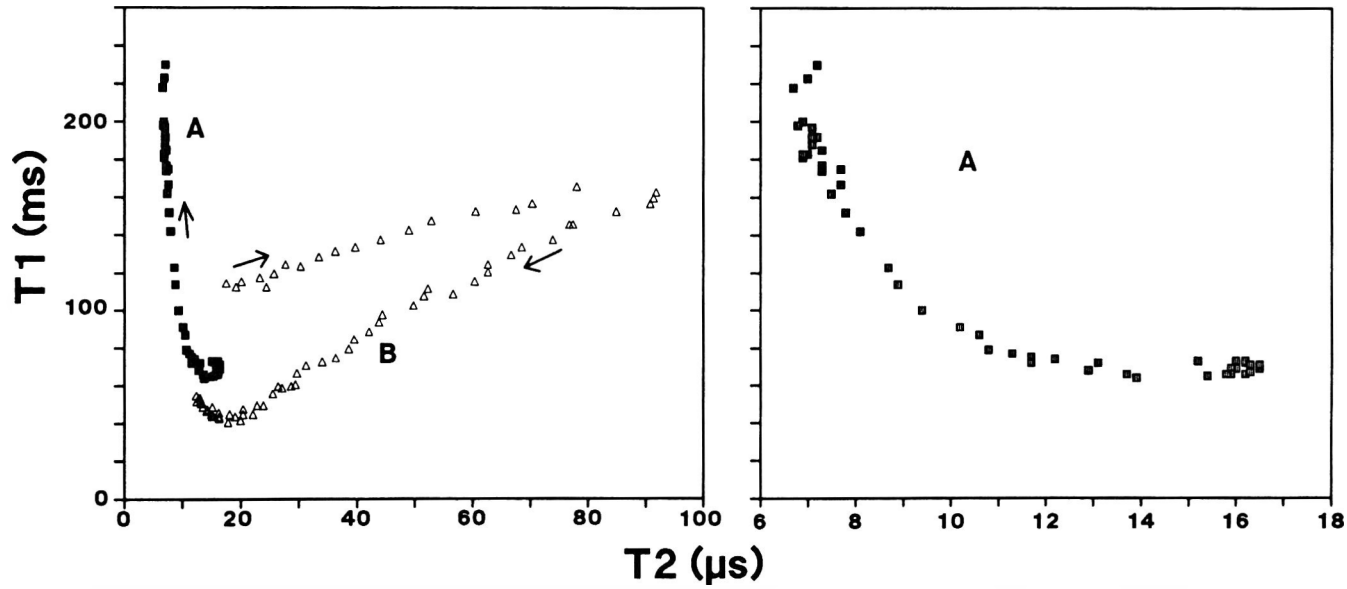

Fig. 5—Characteristic NMR curve for monitoring the freeze-drying of potato samples in the NMR apparatus. Curve A (squares) corresponds to a successfully freeze-dried sample, curve B (triangles) to one in which fusion occurred. The arrows indicate the progression of the freeze-drying process. Curve A is shown in more detail on the right.

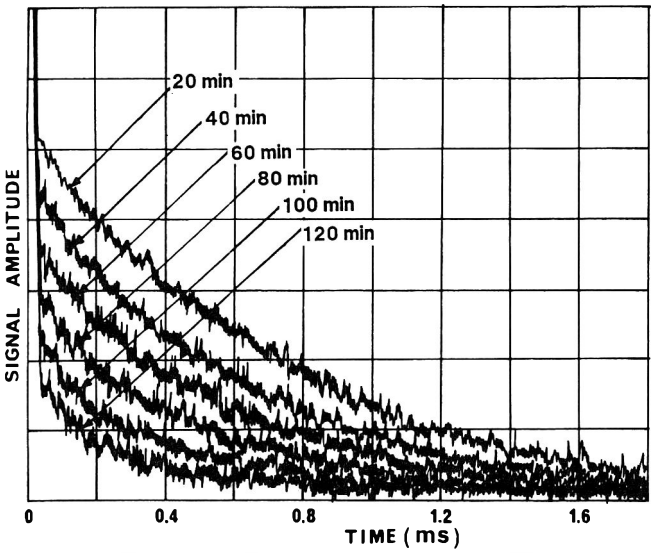

Fig. 6—Evolution of the Free Induction Decay signal intensity as the freeze-drying of a potato sample progressed over a period of 2 hr.

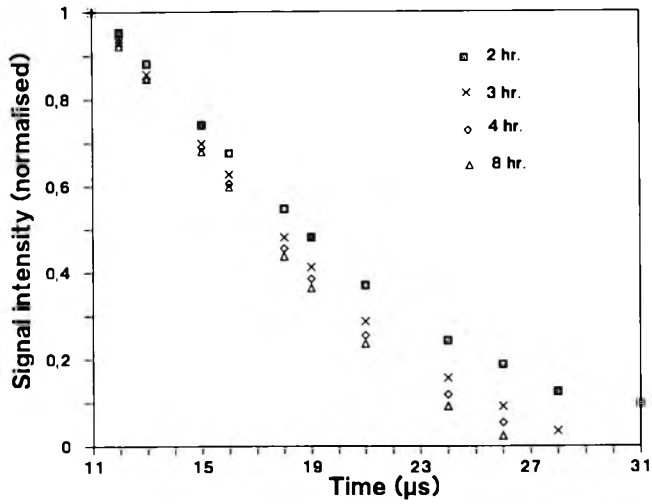

Fig. 7—Normalized signal intensity of the dry layers of freeze-dried potatoes as a function of t, for freeze-drying durations of 2 to 8 hr; t is the delay between the 90° pulse and the measurement.

Table 1—Evolution of water content and T_1 of dry layers of potatoes as a function of freeze-drying time

Freeze-drying time (hours)	Water content (%, wet basis)	T_1 (msec)
2	11.1	90
3	8.5	137
3.5	5.8	213
4	4.7	223
8	4.3	354

desorption theory as they show that during drying, the state of the water in the dry layer changed and the dry layer water content decreased. These results conclusively demonstrated that both desorption, i.e. removal of the residual water from the dry layer, and sublimation occur simultaneously.

In a product with initial water content around 75%, the majority of the protons would be in the frozen core during most of the freeze-drying process. Those protons do not change their characteristics until the end of sublimation because diffusion of unfrozen water is very restricted at the low temperatures of the frozen core. Therefore, while the quantity of water protons

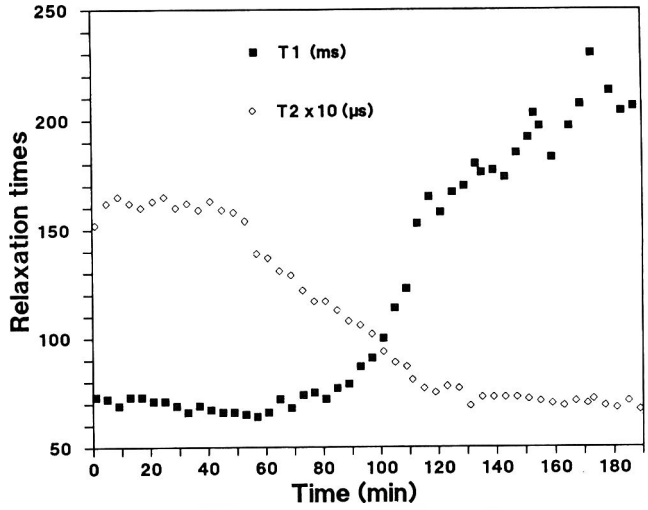

Fig. 8—Evolution of T_1 and T_2 as a function of time during the freeze-drying of a potato parallelepiped in the NMR apparatus.

decreased, their "qualities," or their T_1 and T_2 values, should nevertheless remain unchanged. Figure 8 presents the T_1 and T_2 values used in Fig. 5-curve A plotted as a function of drying time. As expected, the T_1 and the T_2 hardly varied initially. A slight T_1 minimum was nevertheless apparent. For hydrated lysozyme powders, changes in the mobility of water molecules are theorized to be at the origin of the observed minimum for T_1 as a function of temperature (Bryant and Shirley, 1980). The phenomenon observed here may be explained if we assume that the molecular correlation time (τ_c) for which the T_1 relaxation is most efficient (Bloembergen et al., 1948) is close to the τ_c values of relatively mobile water molecules, but distant from the τ_c values of frozen and tightly bound water molecules. Even though desorption of water from the "dry layer" occurs, the proportion of mobile water molecules increased during the sublimation period. This would explain the slight decrease in T_1 observed in Fig. 8. Soon after complete elimination of the frozen core, T_1 increased sharply (see Fig. 2) due to desorption of the last remaining mobile water molecules from the "dry layer".

T_1 increased abruptly with drying time between 90 and 120 min (see Fig. 8); afterwards this increase slowed down, just as did the decrease in T_2. Since the slope of T_1 vs water content increased with decreasing water contents (see Fig. 2), this deceleration could be explained by the water content beginning to decrease more slowly, that is, by a change in drying kinetics. It seems logical to hypothesize that after the elimination of the frozen core the drying rate stays relatively high until the desorption of the more mobile water is complete. This point, at which the drying rate clearly decreases, may have important technological repercussions.

CONCLUSION

ANY IMPROVEMENT in modeling of freeze-drying kinetics would require a description of the mechanisms of desorption of water remaining in the "dry" part of the product. That desorption and sublimation occur simultaneously is a significant finding. Whatever the future developments in modeling the process, NMR is well suited to the monitoring of freeze-drying. Estimations of T_1 and T_2 using rapid methods can be useful for detection of sublimation end points which afford obvious technological and economic advantage. Moreover, a change in drying kinetics, which occurs after the sublimation end point, can be detected, as well as the fusion of the frozen core, an important freeze-drying anomaly. However, suitable sampling devices need to be developed prior to industrial application of these results. —Continued on page 1728

Color Development in a Model System During Frying: Role of Individual Amino Acids and Sugars

M.A. ROE and R.M. FAULKS

ABSTRACT

The color of fried potato products is limited by reducing sugars, but can be affected by free amino acids. Filter paper discs saturated with solutions of amino acids in combination with glucose, fructose and sucrose were fried in oil to investigate color formation. Four phases of color development were identified; equilibrium, lag, rapid and slow phase, corresponding to the water content of the system. Lysine, γ-aminobutyric acid and glycine produced most color and glutamic acid least. Fry color of glucose and fructose systems was very similar, but sucrose systems produced less color. Glucose and fructose systems were not affected by pH changes, but there was a slight effect on sucrose systems.

Key Words: frying, color, amino acids, sugars

INTRODUCTION

THE CONTENT of reducing sugars and amino acids has been shown to have an effect on the color of fried potatoes (Habib and Brown, 1956; Schallenberger et al., 1959). The disappearance of amino acids has been found to accompany the disappearance of reducing sugars during browning, indicating that the color produced was probably due to the Maillard Reaction between reducing sugars and amino acids (Townsend and Hope, 1960; Fitzpatrick et al., 1965). Excessive browning during frying produces an undesirable color and a bitter taste, which are unacceptable in fried potato products. The potato processing industry uses reducing sugar levels as a predictive test of the suitability of material for processing, since reducing sugars are normally the limiting factor in color development (Dahlenburg, 1982; Marquez and Añon, 1986). Relationships between fry color and reducing sugar content have varied considerably and this variation has been ascribed to the differences in free amino acid concentrations in the potato (Habib and Brown, 1957; Hope et al., 1960). The free amino acid pool of potato tubers varied with different levels of nitrogen fertilisation (Eppendorfer, 1978; Hoff et al., 1971; Rexen, 1976) and with the effect of post harvest storage (Fitzpatrick and Porter, 1966). More recently changes in the relative proportions of the sugars and free amino acids present have been reported in potatoes grown under different nitrogen regimes (Roe et al., 1990).

Of the many investigations of the Maillard Reaction most have measured color produced in solutions. Where browning has been investigated during frying, filter paper discs impregnated with potato juice or solutions of amino acids and sugars have been used (Townsend and Hope, 1960; Dahlenburg, 1982; Nam and Kim, 1984; Leskowiat et al., 1990), however such systems have not been used to investigate the role of individual amino acids and sugars. The color produced during frying may not be entirely due to the Maillard Reaction between reducing sugars and amino acids. Sucrose can have an effect on color development if conditions are favorable for hydrolysis. Schallenberger et al. (1959) and Townsend and Hope (1960) found that conditions favoring hydrolysis of sucrose could occur during frying, although sucrose alone has not correlated well with fry color. Caramelization of sugars can occur simultaneously with the Maillard Reaction and this may contribute slightly to total color. Buera et al. (1987) found that color due to caramelization was not generally important when compared with Maillard browning. Other factors such as pH (Wolfrom et al., 1974; Ashoor and Zent, 1984; Buera et al., 1987), buffer ions (Burton and Mcweeny, 1963; Saunders and Jervis, 1966) and water content (Eichner and Karel, 1972) have also been shown to affect color development. However Nam and Kim (1984) reported that the effects of pH were obscured at normal frying temperatures (ca 180°C). Recent work where amino acids were ranked in order of browning potential has shown that although browning may be influenced by physical conditions, the browning potential of individual amino acids was relatively unaffected (Ashoor and Zent, 1984; Ames, 1986).

Because of the variable ratios and total amounts of sugars and amino acids present in potatoes and the lack of data on high temperature-short time processes, model frying experiments were carried out to identify factors that influence color development. Our objective was to determine the degree of browning in fried filter paper discs saturated with solutions of amino acids and sugar. We also wanted to determine the relative contributions and influence of specific amino acids, sucrose and pH.

MATERIALS & METHODS

AMINO ACIDS (γ-aminobutyric acid, arginine, asparagine, aspartic acid, glutamic acid, glutamine, glycine, lysine, phenylalanine and valine) and sugars (D-glucose, D-fructose and sucrose) were analytical grade and were obtained from a range of suppliers. Analytical grade buffer salts, disodium hydrogen orthophosphate and sodium dihydrogen orthophosphate were obtained from BDH (Poole, Dorset, England). Glass distilled water was used throughout to make up the solutions. Filter paper discs, Whatman No 17 (5.5cm dia.) were used without prior treatment. Frytol vegetable oil (commercial oil blend supplied by Herbert Freeston Ltd., Norwich, Norfolk, England) was used for frying.

Stock solutions (0.2M) of amino acids and sugars were prepared in 0.1M phosphate buffer in the range pH 4–8. To prepare working solutions, equal aliquots of sugar and amino acid solutions were mixed and diluted to 0.02M with the appropriate 0.1M phosphate buffer and the final pH adjusted by the addition of 0.1M HCl or 0.1M NaOH. The working solutions were used to saturate weighed filter paper discs, excess solution was wiped off with absorbent paper. Duplicate saturated wet or freeze-dried discs were placed between two pieces of thin wire mesh with 1 cm^2 openings and immediately fried in oil at 180°C for 10 to 120 sec. Filter paper discs saturated with buffer were fried as controls. Immediately after frying, the filter paper discs were plunged into liquid nitrogen to prevent further evaporative loss of water and placed in small covered petri dishes to warm up to room temperature prior to weighing. The color of the fried filter paper discs was measured on a Hunter Labscan (HunterLab, Reston, VA) using the Hue angle (θ) (a measure of the relative intensities of color components) as defined by the manufacturers. After color measurements the fried filter paper discs were dried to constant weight at 103°C in a fan assisted oven. From the weights, the amount of water present in the filter paper and the fat uptake were calculated.

RESULTS & DISCUSSION

The choice of amino acids and sugars was based on those present in greatest amounts as free compounds in potato tuber

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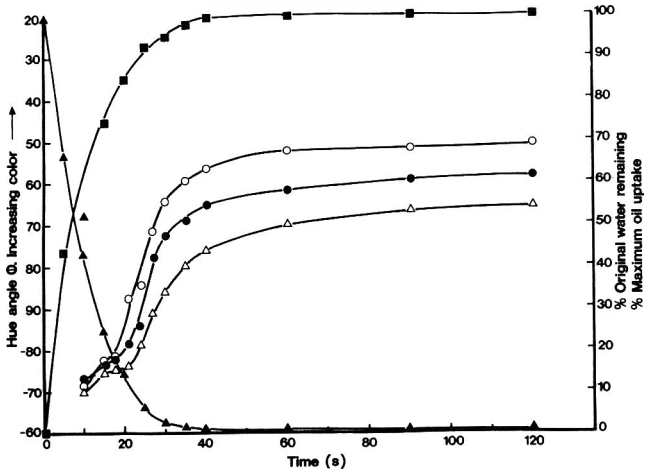

Fig. 1—Development of color, % of original water remaining and % of maximum oil uptake for systems of 0.02M glucose with 0.02M lysine, glutamine and glutamic acid at pH 6.0. ▲ % of original water remaining; ■ % of maximum oil uptake; ○ 0.02M glucose/0.02M lysine; ● 0.02M glucose/0.02M glutamine; △ 0.02M glucose/0.02M glutamic acid.

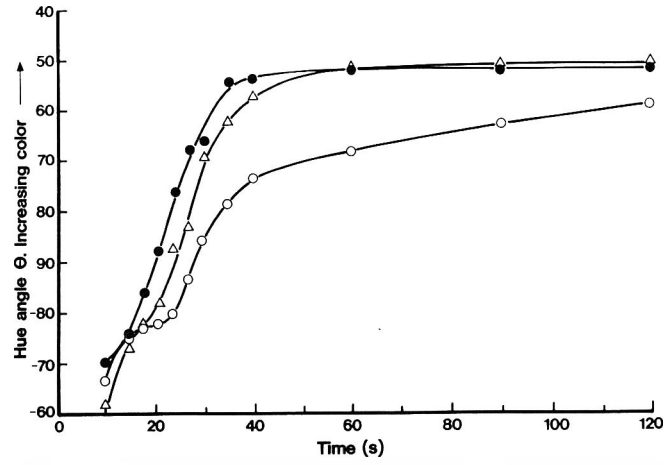

Fig. 2—Development of color in systems of 0.02M glucose, fructose and sucrose with 0.02M γ-aminobutyric acid at pH 6.0. ● 0.02M glucose, △ 0.02M fructose, ○ 0.02M sucrose.

tissue (Davies, 1977; Burton, 1966). The concentration of sugars was similar to that found in the expressed sap of stored tubers (0.36% w/v) but the concentration of individual amino acids was much higher than that normally found (Synge, 1977). A much higher concentration of amino acids was used to obtain a molar ratio of 1:1, sugar:amino acid, the stoichiometry of the initial stage of the Maillard Reaction (Ellis, 1959). Although not physiologically representative this ratio was used also to maximize color production by avoiding limitations in the concentration of either component. This helped enable identification of those systems with the greatest potential to produce colored products. The pH (6.0) corresponded to the average pH for potato tuber sap which has been reported to vary between pH 5.5 and 6.5 (Burton, 1966).

The general shape of the curves relating color development, accompanying water loss and oil uptake were similar for all systems. Examples of the curves obtained with D-glucose and some amino acids are shown in Fig. 1. Clearly, color development had four distinct phases. First, a slight but rapid increase in color up to about 15 sec, then a lag phase of variable duration of 15 to 22 sec. The third phase was a rapid increase in color between 22 and 40 sec and finally a plateau between 40 and 120 sec where little additional color was formed.

In the first phase, although the color appeared to be increasing rapidly, this was due to an effect of oil on the surface of the filter paper which changed the optical characteristics. Any changes after this initial period of equilibriation were caused by true changes in color. The lag phase occurred once the paper was oil saturated, but no color developed because of the high water content. Perhaps there was sufficient steam generation during this high moisture phase to prevent the paper temperature rising much above 100°C, at which the Maillard Reaction is relatively slow (Ellis, 1959).

The onset of rapid color development (3rd phase) occurred once about 20% of the original water was left. This would cause a fivefold increase in concentration of the substrates and the reduced water content probably allowed the temperature of the paper to rise, greatly increasing the rate of the Maillard Reaction. The beginning of the fourth phase, during which no further color developed, coincided with the point at which almost none of the original water was left. The lack of water to solvate substrates may well be the reason that no further color developed, however it was not clear whether all reactants had been consumed. Saturated filter paper discs which were freeze-dried before frying, developed color, although to a lesser extent, despite the lack of water. This could have been a result

Table 1—Mean Hue Angle (θ) of duplicate fry tests of amino acid-sugar solutions after frying for 2 min (In order of decreasing fry color)

	Glucose[a]	Fructose[a]	Sucrose[b]
Lysine	50.52	50.55	61.31
γ-Aminobutyric acid	52.39	52.24	61.60
Glycine	54.90	55.22	64.21
Glutamine	57.23	59.47	60.29
Aspartic acid	58.50	62.72	62.49
Arginine	59.11	59.92	69.98
Valine	59.37	61.22	72.90
Phenylalanine	61.13	62.24	75.56
Asparagine	62.30	63.11	74.24
Glutamic acid	65.24	64.24	67.39

[a] Columns with different superscripts are significantly different ($P<0.01$) using paired t-tests.

Table 2—Mean Hue Angle (θ) of sugar-glycine solutions at pH 4.0, 6.0 and 8.0 after frying for 2 min

	pH ± SD mean[a]		
	4.0	6.0	8.0
Glucose/Glycine	55.30 ± 2.03	54.57 ± 0.39	54.31 ± 1.55 NS
Fructose/Glycine	54.87 ± 0.59	56.41 ± 1.99	58.07 ± 1.87 NS
Sucrose/Glycine	57.78 ± 1.99	65.83 ± 0.89	74.54 ± 0.51 $p < 0.01$

[a] Significance of pH assessed by oneway analysis of variance. NS = not significant.

of the high concentration of reactants in the dry system, caramelization of sugars, or reactions between components in the oil and the sugars and/or amino acids (Kwon et al., 1965; Montgomery and Day, 1965). These findings agreed with the known Maillard mechanism for non-enzymic browning, where ketoseamines are formed in low yields in dilute solutions and in maximum yields in mixtures with about 18% water content (Reynolds, 1970). Discs saturated with blank solution (phosphate buffer pH 6.0) or individual sugar solutions produced no color ($\theta = -80.0$ after 120 sec). The phases of color development appeared to be related to the rate of heating, amount of water (concentration of reactants) and the course of the Maillard Reaction under changing conditions.

Comparison of the browning effects of the different sugars (Table 1) showed that both D-glucose and D-fructose gave similar and significantly higher color than sucrose with each amino acid. Very little difference occurred in the order of ranking between the amino acids with D-glucose and with D-fructose. Lysine, γ-aminobutyric acid and glycine were the highest browning amino acids and may therefore be the most important in browning of potato chips. With D-glucose, the lightest colors formed were similar to the darkest color formed with sucrose.

A representative plot of comparitive rates of color development for different sugars is given in Fig. 2. Both D-glucose

and D-fructose reacted as expected but sucrose, which does not participate in the Maillard Reaction as such, showed much less color production in the third (rapid) phase. Additionally, with sucrose there was no plateau and color continued to be generated up to 120 sec. The color reaction therefore continued, unlike the Maillard Reaction with reducing sugars. The reasons are unclear but it may have been due to the hydrolysis of sucrose to reactive monomers, caramelization or other unspecified reactions with components in the oil at 180°C in the virtual absence of water (Schallenberger et al., 1959; Townsend and Hope, 1960).

A comparison of the effects of pH on color development with selected amino acids showed no significant difference with D-glucose or D-fructose but pH had a significant effect on color development with sucrose (Table 2). For the reaction between sucrose and glycine the greatest final color ($\theta = 58$) occurred at pH 4.0 and the least ($\theta = 76$) at pH 8.0. This suggested that at least some slight hydrolysis of sucrose might occur and the resulting reducing sugars may have participated in the Maillard Reaction. However, the change in pH from 4.0 to 8.0 could result in a 10^4 decrease in reaction rate since the hydrolysis of sucrose is an acid-catalyzed process. The rate-limiting step would be hydrolysis rather than the Maillard Reaction. The hydrolysis rate could be expected to change by 10^4 between pH 4.0 and 8.0 with resulting changes in color development of the same order. However, this did not occur probably because of the limiting amount of water in the system. Thus sucrose contributed only marginally to color development through its hydrolysis products.

Preliminary studies were undertaken using mixtures of two or more amino acids with D-glucose. In those systems, a mean fry color was obtained which could be predicted from results of the single amino acid - D-glucose systems. No synergistic or depressive effects were noted in such mixed systems, supporting the findings of Nafisi and Markakis (1983).

CONCLUSIONS

DIFFERENCES occurred in the degree of browning between different amino acids and sugars under frying conditions. There was no significant difference in color formation of glucose-amino acid or fructose-amino acid systems, but sucrose systems produced much less color. In potatoes amino acids are present in excess of sugars and the difference in color formed by different amino acids was relatively slight. Thus there were no indication of particular amino acids reacting preferentially. Determination of the amount of free amino acids present in potatoes would not likely improve the predictability of fry color (Roe et al., 1990).

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Simultaneous Heat Processing of High- and Low-Acid Foods in Semirigid Containers—A Theoretical Analysis

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ABSTRACT

A one meal-set of military ration consists of heat sterilized, high- and low-acid foods in semirigid containers. These foods are processed separately because of large differences in lethality requirements. We examined the feasibility of simultaneously processing a meal-set consisting of chili con carne, white rice, and sliced peach in syrup to minimize costs. Simultaneous processing was not possible by adjusting initial temperatures according to computer-simulation using food temperature data collected experimentally. However, it was possible when the container for peaches was insulated properly with expanded polystyrene. Proper insulation thickness was slightly dependent on z value of the target factor.

Key Words: acid foods, semirigid containers, packaging, computer-simulation, thermal processing

INTRODUCTION

HEAT STERILIZED foods in semirigid, plastic containers have been accepted by consumers because of their easily recognizable package profiles and of their shelf life stability. Thermal processes for foods in those containers are described in several articles (Dahlgren, 1985; Wachtel, 1987). A tray-set of different, thermally processed foods in semirigid containers has been served as a complete meal for military feeding due to convenience for mass feeding. High- and low-acid foods usually are included in one set. These packaged food components are processed separately because of greatly different target process lethalities. The simultaneous processing of one complete meal-set could greatly reduce production costs of military rations. The objective of our work was to analyze feasibility for simultaneously processing high- and low-acid foods packed in semirigid containers.

MATERIALS & METHODS

A MEAL-SET consisting of chili con carne, white rice, and peach slices in syrup was used. The first two are low acid foods and the remaining high acid food. Two approaches for accomplishing simultaneous processing of the three foods were examined. The first was to adjust initial food and retort temperatures. The second was to apply a layer of insulation to containers for peach slices, which required the least process for lethality. A computer program, a modified version of one previously developed for evaluation of thermal processes (Hayakawa, 1977), was used. The target process lethality and the heat transfer parameters of packaged foods were required for this. They were obtained as described below.

Thermal death time characteristics

A target sterilizing value, F_p, of each food was obtained from the experimental temperature data for each food, which has been subjected to a proper thermal process (Table 1, data provided by U. S. Army Natick RD&E Center). All heat penetration experiments were done in a still retort manufactured by the Berlin Food Processing Equipment Co. The heating medium was hot water pressurized with air.

The following equation calculates the sterilizing values (Ball and Olson, 1957):

$$F_p = \int_0^{t_{end}} 10^{(T - T_r)/z}\, dt \qquad (1)$$

Using experimentally determined temperature data, an F_p values were calculated by numerical integration using Simpson's rule (a general method).

It is clear from Eq. (1) that the proper values of z, and T_r, must be chosen to estimate an F_p value. Since chili con carne and white rice are low-acid foods, the target microorganism for thermal processing of both foods is *Clostridium botulinum* spores. Therefore, a standard z value of 10 C° was used. There was no published information available on target microorganisms of peach slices in syrup. However, one reference book (Lopez, 1981) stated vegetative bacteria (e.g., *Bacillus thermoacidurans*, *Clostridium pasteruianum*, *Lactobercillus* sp., *Leuconostoc* sp.), yeasts (only in case of gross underprocessing) or molds (*Byssochlamys fulva*) are the target organisms for thermal processing of high acid foods. The z values of these microorganisms are likely within the range between 4.2 C° and 12 C° according to published reports (Hugo, 1971; Norwig and Thompson, 1986). The following three z values were used for peach slices in syrup, 4.2, 10.0, and 12 C° (10 C° for comparison).

As for T_r, 121.11° C is commonly used to estimate F_p for thermal processes of low-acid foods. It is a common practice to use a T_r below 100° C for high-acid food. However, 121.11° C was used as the T_r for peach slices for the following reasons. First, an F_p based on T_r may be converted easily to another F_p based on another T_r by:

$$F_{p2} = 10^{(T_{r1} - T_{r2})/z}\, F_{p1} \qquad (2)$$

Second, one obtains final conclusions identical to those based on different T_r values when enough significant digits are retained in estimated F_p values (very small values). This may be accomplished through double precision computations.

Heat penetration parameters

Empirical parameters, f and j values, for heat transfer in packaged food were estimated from 6 temperature history curves for each food provided by the U. S. Army Natick RD&E Center. Parameters f and j are related to the slope and intercept of the linear part of a semilogarithmic temperature history, Eq. (3).

$$\log_{10}(|T_a - T|) = -t/f + \log_{10}[j(|T_a - T_i|)] \qquad t > t_e \qquad (3)$$

The f and j values of a semilogarithmic heating curve (f_h and j_h) were estimated by using a computer program developed previously (Hayakawa et al., 1981) after making a correction for come-up heating (Ball and Olson, 1957). The f and j values of the semilogarithmic cooling curve (f_c and j_c) were determined through similar computations. Conduction heat transfer in the food was assumed. Justification for this is presented later.

Temperature response properties of peach slices in syrup

The principles of heat transfer were used to estimate f and j values of peach slices in a syrup package with an applied layer of insulation material. For this, the inside space configuration of the container was approximated by a rectangular parallelepiped (a brick shape) with an identical volume. The parameters f and j of a rectangular parallelepiped, 2L × 2W × 2H, are related to respective heat penetration param-

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Table 1—Processing conditions of selected foods in semirigid containers

	Net weight g	T_o °C	T_1 °C	P_a $\times 10^5 P_a$	t_{up} min	t_b min
Peach slices	227 (136[a])	24.8	104.4	1.082	7.0	16.0
White rice	227	30.3	118.1	2.324	31.0	38.0
Chili con carne	326	34.2	115.6	2.234	11.0	49.0

[a] Drained weight

eters of infinitely wide plates of thicknesses 2L, 2W, and 2H (Ball and Olson, 1957 and Pflug et al., 1965).

$$1/f_p = 1/f_L + 1/f_W + 1/f_H \quad (4)$$

$$j_p = j_L \cdot j_W \cdot j_H \quad (5)$$

The parameters f and j of an infinite plate may be estimated from the analytical formula when thermal diffusivity, α, and Biot number, Bi, are available. For example, the f and j values of an infinite plate of thickness 2H may be estimated by:

$$f_H = H^2 (\ln 10)/(p_{1H}^2 \alpha) \quad (6)$$

$$j_H = j_{Hc} \cos (p_{1H} x/H) \quad (7)$$

Where p_{1H} is the first positive root of the following equation.

$$p \tan p = Bi_H \quad (8)$$

In Eq. (7) j_{Hc} is the j value on the center plane of the plate which may be estimated by (Pflug et al., 1965):

$$J_{Hc} = 2Bi_H \sec P_{1H}/\{Bi_H (Bi_H + 1) + P_{1H}^2\} \quad (9)$$

From Eq. (4) and (6), we obtained:

$$1/f_p = (\alpha/\ln 10) \{(L/p_{1L})^2 + (W/p_{1w})^2 + (H/p_{1H})^2\} \quad (10)$$

where P_{1L} and P_{1W} are the first positive roots of the following equations.

$$P_{1L}: \quad P \tan P = S_L Bi_H \quad (11)$$

$$P_{1W}: \quad P \tan P = S_W Bi_H \quad (12)$$

The central j value, which is required to estimate center temperatures, may be obtained using Eq. (7) and (9).

$$j_p = 8Bi_H^2 S_L \cdot S_W \sec P_{1W} \cdot \sec P_{1H}/[\{S_L Bi_H (S_L Bi_H + 1) + P_{1L}^2\} \{S_W Bi_H (S_W Bi_H + 1) + p_{1W}^2\} \{Bi_H (Bi_H + 1) + p_{1W}^2\}] \quad (13)$$

The values of Bi_H and of first positive roots P_{1L}, P_{1W}, and P_{1H} were determined by substituting an experimentally determined j value into Eq. (13) [only one unknown because of all roots related to Bi_H as shown by Eq. (8), (11), and (12)]. The effective thermal diffusivity α of peach slices in syrup was estimated substituting an experimental f value and the roots into Eq. (10).

The effective thermal conductivity of the food, k, was determined from the definition of α and the estimated density, ρ, and specific heat, C_p, of the food.

$$k = \alpha C_p \rho \quad (14)$$

The food was assumed to be equilibrated with a 15% sugar solution (mass basis) to estimate ρ and C_p. Thus, C_p was obtained from a Mollier chart for sugar solutions (Burke, 1954). The same chart shows the refractive index, n, of the solution. The density ρ was estimated using the following empirical formula (Dickerson, 1968).

$$\rho = 0.00452 (n^2 - 1)/(n^2 + 2) \quad (15)$$

The value of effective Bi is changed when a layer of insulation material is applied to all outside surfaces of a container since an effective, convective surface heat conductance is reduced from h to U, Eq. (16).

$$1/U = 1/h + l_n/k_n \quad (16)$$

$$Bi_{Hn} = UH/k \quad (17)$$

For the present analysis, expanded polystyrene was chosen as an insulation material.

Equation 10 gives a relationship between the value of f for peach slices in an insulated container, f_{pn}, and f of those in noninsulated containers, f_p.

$$f_{pn}/f_p = \frac{[(L/p_{iL})^2 + (W/p_{1w})^2 + (H/p_{1H})^2]}{[(L/p_{1Ln})^2 + (W/p_{1wn})^2 + (H/p_{1Hn})^2]} \quad (18)$$

Similarly, a relationship between j values of peach slices in insulated and noninsulated containers is obtained from Eq. (15).

$$j_{pn}/j_p = \psi (Bi_{Hn})/\psi (Bi_H) \quad (19)$$

where: $\psi (Bi_H) = Bi_H^3 \sec p_{1L} \sec p_{1W} \sec p_{1H}/[\{S_L Bi_H (S_L Bi_H + 1) + p_{1L}^2\} \{S_W Bi_H (S_w Bi_H + 1) + p_{1W}^2\} \{Bi_H (Bi_H + 1) + p_{1H}^2\}]$ (20)

$\psi (Bi_{Hn})$ is defined by replacing Bi_H, P_{1L}, P_{1W}, and P_{1H} by Bi_n, P_{1Ln}, P_{1Wn}, and P_{1Hn}, respectively.

For each assumed thickness of insulation material, Bi_{Hn}, was estimated by Eq. (16) and (17). Then constants P_{1Hn}, P_{1Ln}, and P_{1Wn} were estimated using Eq. (8), (11), and (14) where Bi_H in these equations was replaced by Bi_{Hn}. The values of f_h, f_c, j_h, and j_c applicable to an insulated container package were estimated using Eq. (18) and (19).

Estimation of F_p

A modified version of a computer program developed previously (Hayakawa, 1977) was used to estimate F_p values. This program has an option of specifying either conductive or convective food being processed. Because of an assumption previously stated, the option of heat conductive food was used.

RESULTS & DISCUSSION

TABLE 2 shows the actual sterilizing values calculated from temperature histories provided by the U.S. Army Natick RD&E Center. The F_p of chili con carne and white rice are typical values for low-acid foods. The F_p of peach slices in syrup was highly dependent on the z value used. Since the temperatures of peach slices were below T_r, a smaller z value resulted in smaller values of integrand of Eq. (1) and thus a smaller F_p value.

The examination of the true temperature data revealed that temperatures of all three foods continued to increase during initial cooling periods. This implied the food was conduction heated and cooled since there was no temperature increase during a cooling phase for *convective* heating food. With chili con carne and white rice, this was expected. With peach slices in syrup, there was a relatively small height profile of a container for peach slices, with an average inside height

Table 2—Target sterilizing values (F_p) of three products in semirigid containers

	z Value (C°)	F_p (min)
Chili Con Carne	10.0	7.5
White Rice	10.0	7.5
Peach Slices in Syrup	4.2	4.30×10^{-5}
	10.0	0.0500
	11.9	0.152

Table 3—Average heat penetration parameters of three products in Army ration containers

Parameters	Food		
	Chili Con Carne	White Rice	Peach Slices[a]
f_h (min)	26.49 (0.318)[b]	28.30 (0.348)	18.32 (0.624)
f_c (min)	35.78 (1.11)	59.25 (3.66)	31.35 (3.10)
j_h	1.235 (0.035)	1.375 (0.049)	1.17 (0.085)
j_c	1.209 (0.022)	1.431 (0.280)	1.17 (0.011)

[a] Actual values of packs in uninsulated containers.
[b] Values in pairs of parentheses are standard deviations.

Table 4—Effective inside dimensions (mm) of containers

Container for	Height (2H)	Width (2W)	Length (2L)
Peach Slices	30	70	118
Chili Con Carne	26	92	136
White Rice	30	70	118

of 30 mm, causing the reduced convective flow of the syrup. Based on the above observations, all foods were assumed to be thermally conductive.

Table 3 shows the values of heat penetration parameters and standard deviations. Effective inside dimensions of containers are shown in Table 4. The dimensions of the containers for chili con carne and white rice are given for comparison although they were not required for analysis.

Simulation for adjusted initial food temperatures

The heating time, t_b, should be reduced for the low-acid food and increased for the high-acid food for simultaneous processing. Therefore, the initial temperature, T_o, of white rice and chili con carne were adjusted between 29.4 and 82.2 °C and T_o of peach slices between 1.6 and 15.6 °C. Analyses were performed for two retort temperatures, 110.0 and 121.1 °C. Results are summarized in Table 5.

With retort temperature 110°C, the t_b of both low-acid foods were reduced slightly when T_o were increased from 29.4 to 82.2°C, about a 9% reduction. Note that the t_b of both foods were nearly identical although their f_h values differed. This was due to the fact that the f_c of white rice is considerably greater than the f_c of chili con carne. The larger f_c value resulted in higher food temperature during the cooling phase because of a slower rate of cooling. Therefore, there was a greater cooling phase lethality for white rice as compared to that of chili con carne. This compensated for a lesser heating phase lethality of white rice.

The t_b of peach slices were 15 to 20% of those of the low acid foods depending on the peach z value, 20% for the smallest z value and 15% for the largest z value. There was no significant increase in t_b when the initial food temperature was lowered to 1.6°C.

With the retort temperature of 121.1°C, relative differences in the t_b of the low and high acid foods became less than those at the lower retort temperatures, a 35 to 40% difference. There were relatively large reductions in heating times for low-acid food when initial temperatures were increased (20% reduction). However, there were no significant increases in required heating times for the high-acid food with lowered initial temperature. The z values did not significantly influence the high-acid food heating times. Clearly, from Table 5, the simultaneous processing of all foods is not possible by adjusting the retort or initial temperatures.

Simulation for insulated high-acid food containers

The simulation required the effective thermophysical property values of peach slices in syrup. The estimated values are:

$$\alpha = 2.07 \text{ mm}^2/\text{sec} \quad \text{(a mean of six values, } \pm 10\%)$$

$$C_p = 3.81 \text{ J/(gC°)} \qquad n = 1.356$$

$$\rho = 0.001060 \text{ g/mm}^3 \text{ k} = 0.00836 \text{ W/(mmC°)}$$

The thermal conductivity of expanded polystyrene is 4.19×10^{-5} W/(mm C°) (Anonymous, 1988). The effective thermophysical property values of peaches in syrup and the insulation material were used to convert the experimentally determined f and j values of peach slices in a noninsulated container to those in an insulated container as previously described.

Proper thicknesses of expanded polystyrene layer on the peach container were determined through iterative computations. The following two different sets of results were obtained: (1) Different initial temperatures yielding virtually identical, required heating times (less than

Table 5—Predicted heating times of three rations of different T_o and different T_1[a]

	T_1 = 110°C				T = 121.11°C		
Food[b]	T_o (°C)	z (C°)	t_b (min)	Food[b]	T_o (°C)	z (C°)	t_b (min)
C	29.44	10.0	137.0	C	29.44	10	44.74
	37.78		135.7		37.78		43.64
	65.56		130.1		65.56		38.97
	82.22		124.7		82.22		34.87
R	29.44	10	137.0	R	29.44	10	45.65
	37.78		136.6		37.78		44.48
	65.56		130.6		65.56		39.50
	82.22		124.9		82.22		35.11
P	1.67	4.2	27.14	P	1.67	4.2	17.69
	4.44		26.99		4.44		17.61
	10		26.68		10		16.67
	15.56		25.62		15.56		16.49
P	1.67	10	20.91	P	1.67	10	15.62
	4.44		20.70		4.44		15.44
	10		20.27		10		15.06
	15.56		19.76		15.56		14.64
P	1.67	12	21.86	P	1.67	12	16.26
	4.44		21.66		4.44		16.08
	10		21.22		10		15.67
	15.56		20.77		15.56		15.26

[a] Cooling water temperature was assumed to be 12°C.
[b] C = chili con carne, R = white rice, P = peach slices.

Table 6—Simulation for insulated peach containers with three different peach z values and with different or same initial food temperatures[a]

Peach −z (C°)	T_o Different[c]	T_o Same[c]	C (°C)	R (°C)	P (°C)	I_m (min)	Peach Parameters[2] f_h (min)	f_c (min)	j_h (−)	j_c (−)	t_b C (min)	R (min)	P (min)
4.2	0		18.3	26.7	27.2	1.23	60.0	72.8	1.054	1.077	49.10	49.07	49.08
4.2		0	23.9	23.9	23.9	1.23	60.0	72.8	1.054	1.077	48.45	49.43	49.47
10.0	0		18.3	26.7	23.1	1.65	74.0	86.2	1.044	1.064	49.10	49.07	49.02
10.0		0	23.9	23.9	23.9	1.65	74.0	86.2	1.044	1.064	48.45	49.43	48.66
12.0	0		18.3	26.7	23.9	1.62	73.1	85.3	1.045	1.065	49.10	49.09	49.03
12.0		0	23.9	23.9	23.9	1.62	73.1	85.3	1.045	1.065	46.45	49.43	49.03

[a] Column headings: C—chili con carne, R—white rice, P—peach slices in syrup; T_1 = 120°C and T_w = 11.7°C for all processes.
[b] Heat penetration parameters for peach slices in syrup in an insulated container. See Table 3 for the other foods.
[c] Different = different initial temperatures; same = same initial temperatures.

0.1% difference); and (2) The same initial temperature yielding slightly different, required heating times.

The estimated initial food temperatures, insulation thickness and processing times (heating time) are shown in Table 6. With the peach z value of 4.2 C°, virtually identical processing times (heating times) were obtained when the initial food temperatures were 18.3°C for chili con carne, 26.7 for rice, and 27.2 for peach slices. When initial temperatures of all foods were equal to 23.9°C, slightly different processing times at 120°C resulted, 49.5 (min) for peach slices, 48.2 for chili con carne, and 49.4 for rice. Therefore, the three foods may be processed simultaneously for 50 min at 120°C, (less than 1 min difference in estimated t_b.

When the z value for peach was 10 °C, the required insulation thickness was 1.65 mm. Required initial food temperatures for virtually similar processing times were 18.3°C for chili con carne, 23.1 for peach, and 26.7 for rice. When the initial temperatures of all foods were set to 23.9°C, the estimated processing times were 48.5 min for chili con carne, 48.7 for peach, and 49.4 for rice. Therefore, all three foods with 23.9°C initial temperatures may be processed for 50 min at 120 °C, identical to the least peach z process.

With the peach z value of 12 C°, the required insulation thickness was 1.62mm. The required initial temperature for nearly the same processing times for chili con carne and rice were identical to those of the two previous peach z values. The initial temperature of peach, 23.9°C was slightly above that for the peach z of 10 C°. When the initial temperature of all foods was 23.9°C, the required processing time of peach was 49.0 min, slightly longer than that for the peach z of 10C°. Again, all foods could be processed simultaneously for 50 min at 120°C when their initial temperature is 23.9°C.

The above analysis assumes no contact resistance between the insulation layer and outside surface of the peach container. A thinner layer of insulation is required when there is significant contact resistance. The time invariable thermal conductivity of expanded polystyrene during processing was another assumption. A thicker insulation layer is required if there is an increase in the thermal conductivity owing to moisture permeation. Thus, a closed cell structure is necessary for insulation.

The required insulation thickness for the two larger peach z values was about 0.4 mm greater than that for the smallest z value. However, the insulation thickness for the largest peach z value (12 C°) was not greater than that for the peach z value of 10 C° (0.04 mm less). This could be due to interactive influence of heat penetration parameters on process lethalities.

The application of insulation material to the peach container introduces additional statistical variability in the process lethality. Therefore, a brooder safety factor is required for simultaneous processing of the three foods.

NOMENCLATURE

Bi	Biot number = $h\ell/k$.
Bi_H	= hH/k.
C_p	Specific heat of food.
f	Slope index of semilogarithmic temperature history curve.
F_p	Sterilizing value.
H	One-half of effective inside height of container whose shape is approximated by a brick shape.
h	Overall heat transfer from heat exchange medium to food surface.
I_n	Insulation thickness.
j	Empirical heat penetration parameter related to the intercept of semilogarithmic temperature history curve.
k	Thermal conductivity.
L	One-half of effective inside side dimension of container whose shape is approximated by a brick shape.
ℓ	Half thickness of a plate.
n	Reflective index.
P_a	pressure
p_1	First positive root of p tan p = B_1.
S_L	= L/H.
S_W	= W/H.
T	Temperature.
T_a	Temperature of surrounding heat exchange medium.
T_i	Food temperature at the beginning of heating phase (identical to T_o) or at the beginning of cooling phase.
T_o	Food temperature at beginning of thermal process.
T_I	Holding temperature of heating medium.
T_W	Temperature of cooling water.
t	Time.
U	Overall coefficient for heat transfer through insulated container wall.
W	One half of effective, inside side dimension of container whose shape is approximated by a brick.
x	Location variable. Distance measured from central plane of infinite plate.
z	Slope index of thermal death time curve (C°).
α	Thermal diffusivity.
ρ	density.
ψ ( )	Function defined by Eq. (20).
Subscript	
b	Denotes process time.
c	Cooling phase of process.
end	End of process (end of Cooling phase).
H	Related of plate of thickness 2H.
h	Heating phase of process.
ℓ	Related to time span of initial curvilinear portion of heating or cooling curve.
L	Related to plate of thickness 2L.
n	Insulation.
P	Rectangular parallelepiped.
r	Reference value.
up	Come-up heating.
W	Related to plate of thickness 2W.
1,2	First and second values, respectively.

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Can Material Influence on the Performance of Rotating Cookers

SHAHED ZAMAN, ENRIQUE ROTSTEIN, and KENNETH J. VALENTAS

ABSTRACT

The rotational performance of ETP (electro-tin-plated) and TFS (tin-free-steel) cans was studied in pilot and commercial cookers, and in a cooker simulator. TFS cans rotated less than ETP cans in both commercial and pilot cookers. Commercial cookers showed less can rotations than corresponding pilot scale experiments. The simulator predicted an exponential decay in lethality with decreased can rpm. Polymer coated cans conditioned the cooker surface and the extent depended on contact time. Heat transfer depended on can rotation and could be predicted with a suitable correlation. The correlation was used to predict lethality performance as well as exit temperature of processed foods.

INTRODUCTION

AXIAL ROTATION of cans which have been filled leaving a headspace is a very effective means of increasing heat penetration. When the food material is highly viscous effectiveness of this procedure may be reduced. Therefore, it is important to understand the limitations of such a process.

Berry et al. (1979) carried out a pioneering study where cream style corn (CSC), a highly viscous non-Newtonian food material, was processed in a Steritort (FMC Corp., Canning Machinery Div., San Jose, CA). The study indicated the influence of headspace, reel speed and product consistency on sterilization performance. The Steritort is pilot-scale equipment, corresponding to the full scale Sterilmatic cooker (FMC Corp.)

In the Steritort and Sterilmatic cans are indexed into pockets on a revolving reel. As the reel rotates the cans become free-rolling; this provides can rotation and resulting agitation. Recently, the amount of rotation has been found sensitive to lid material or finish. Rotstein et al. (1988) provided quantitative data showing that electronically tin plated (ETP) cans rotated more than tin free steel (TFS) cans, when processed in the same cooker. They provided some early analysis of the lethality implications of a decrease in can rotation. Saguy and Kiplocks (1989) designed a device to measure can revolution within a Steritort and found significant rotation differences between ETP and 2-piece and 3-piece TFS cans. Rasmussen et al. (1991) patented a mechanical rotation counter which can be used in Steritorts or Sterilmatics. This device was reported by Rotstein et al. (1988).

A decrease in lethality due to rotation decrease could lead to unsafe conditions. The objective of our work was to further quantify the decrease in rotation, both in pilot and commercial cookers. On the basis of these data, we analyzed the effect on heat transfer and lethality due to changes in instantaneous rpm and total rotations. Further, we wanted to clarify the constraints when scaling up from a pilot to a commercial unit. The study focused on processing CSC because its viscous properties made the process highly rotation-controlled.

THEORETICAL CONSIDERATIONS

Can rotation and reel rotation

Consider a can of diameter D, which rotates on a shell of diameter D_r, driven by a reel rotating at N_r rpm (Fig.1). The maximum instantaneous can rpm, N°, corresponds to the non-slip situation, in which case the linear velocity of the can surface equals the peripheral velocity of the reel:

$$\pi N^\circ D = \pi N_r D_r \quad (1)$$

resulting in:

$$N^\circ = N_r D_r / D \quad (2)$$

The time for one reel rotation is:

$$t_1 = 1/N_r \quad (3)$$

If the reel arc over which can rotation occurs is θ (Fig. 2), the time during which a can rotates for every reel rotation will be:

$$t_{GO} = (1/N_r)(\theta/360) \quad (4)$$

The cans will not rotate the balance of the time between t_{GO} and t_1, i.e.:

$$t_{STOP} = t_1 - t_{GO} \quad (5)$$

On the basis of the above, the ideal maximum number of rotations that accumulate over a processing time t will be:

$$n^\circ = N^\circ\, t\, \theta/360 \quad (6)$$

and the total can rotation time is:

$$t_{rot} = t_{GO}\, N_r\, t \quad (7)$$

In practice, several factors will result in an actual instantaneous rpm $N < N^\circ$ and an accumulated number of rotations $n < n^\circ$. There will be some slip on surfaces, which will depend on the

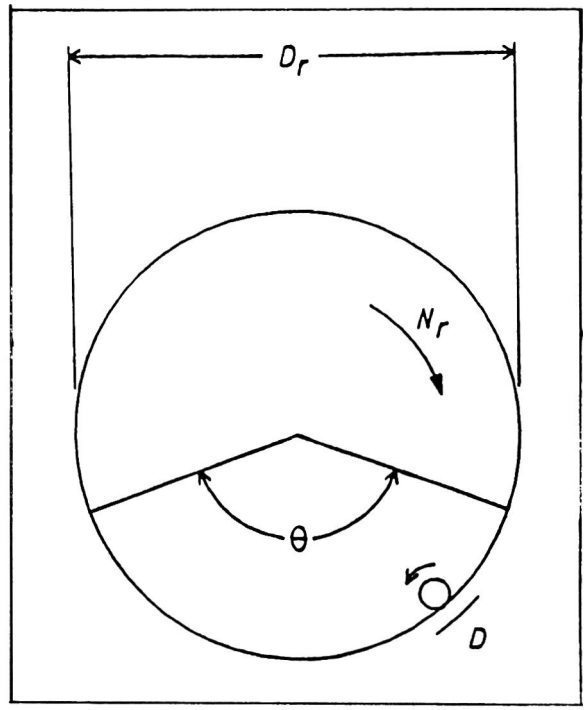

Fig. 1—Geometrical variables in a rotary cooker.

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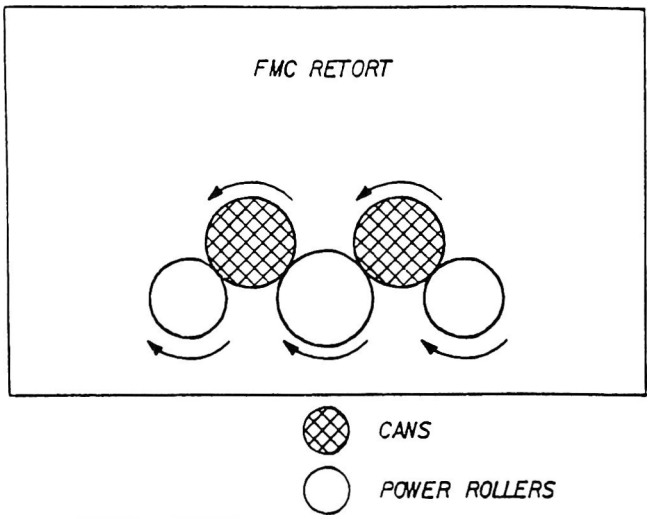

Fig. 2—Cooker simulator

nature and characteristics of the can and reel surfaces. There may be a different pocket angle or other mechanical details. A different state of wear of the surfaces could occur as well as other differences from one cooker to another. Thus:

$$N = fN° \tag{8}$$

$$n = f\,N°t\theta/360 \tag{9}$$

where $f<1$ is a coefficient accounting for the above factors. By the same reasoning, θ will probably be different for different can materials and cookers. The average frequency of can rotation, N_{av}, is:

$$N_{av} = f\,N°\,\theta/360 \tag{10}$$

The heat transfer problem

Using the equation of change in a suitable dimensionless form (Bird et al., 1960) it is possible to predict the shape of a correlation for heat processing of viscous materials in axially rotating cans. Taking into account that cans rotate and cease rotation periodically, one should expect that the heat transfer correlation describing the action would be of the form:

$$Nu = Nu_{nc} + Nu_{fc} \tag{11}$$

where the natural convection Nusselt number would be given by a correlation:

$$Nu_{nc} = \delta_1 (Gr')^{\delta_2} (Pr')^{\delta_3} \tag{12}$$

and the forced convection Nusselt number:

$$Nu_{fc} = \alpha_1 (Re')^{\alpha_2} (Pr')^{\alpha_3} (D/L)^{\alpha_4} \left(\frac{H}{L}\right)^{\alpha_5} (Fr')^{\alpha_6} \tag{13}$$

Notice that a correlation of the shape of Eq. (11) was presented by Rao et al. (1985). In the equations above the dimensionless number D/L accounts for can geometry; (H/L), for head space. Nu', Re', Pr', Gr' and Fr' are the familiar Nusselt, Reynolds, Prandtl, Grashof and Froude numbers (defined in Nomenclature). The generalized viscosity for rotation flow of a power-law fluid is:

$$\eta = KN^{m-1} \left(\frac{4\pi}{m}\right)^{m-1} \tag{14}$$

Earlier work (Rotstein et al., 1988) showed that use of the Froude number did not improve the correlation.

Equations (12) and (13) result from heat transfer to a can and, as such, are based on can size and rpm. Both Rao et al. (1985) and Rotstein et al. (1988) used reel rpm, N_r, to account for rotation. This was done on the basis that:

$$N_r = k_s N \tag{15}$$

where k_s is a function of can material and size and product characteristics. In practice, it is easy to monitor reel rpm while can rpm are difficult to monitor on a routine basis. Thus, it is important to show how the heat transfer correlation can be written in terms of N_r instead of N. Equations (2) and (8) provide a way to redefine the dimensionless numbers in terms of reel rotation, leading to reel rpm-based Re, Pr and Gr. (New definitions are shown in Nomenclature).

The Nusselt numbers can now be written:

$$Nu_{nc} = \delta' Gr^{\delta_2} Pr^{\delta_3} \tag{16}$$

where

$$\delta' = \delta_1 f^{(1-m)(2\delta_2-\delta_3)} \tag{17}$$

and

$$Nu_{fc} = \alpha' Re^{\alpha_2} Pr^{\alpha_3} (D/L)^{\alpha_4} \left(\frac{H}{L}\right)^{\alpha_5} \tag{18}$$

where

$$\alpha' = \alpha_1 f^{(2-m)\alpha_2-(1-m)\alpha_3} \tag{19}$$

Both α_1 and δ_1 are functions of f. Furthermore, in each cycle of the reel forced convection takes place during t_{GO} and natural convection during $(t_1 - t_{GO})$. Therefore the Nusselt number will be given by:

$$Nu = \left(1 - \frac{\theta}{360}\right) Nu_{nc} + \frac{\theta}{360} Nu_{fc} \tag{20}$$

In practice, experiments in a given cooker will yield a correlation based on Eq. (11), taking into account Eq. (16) through (20):

$$Nu = \delta\, Gr^{\delta_2} Pr^{\delta_3} + \alpha\, Re^{\alpha_2} Pr^{\alpha_3} (D/L)^{\alpha_4} \left(\frac{H}{L}\right)^{\alpha_5} \tag{21}$$

where

$$\delta = \left(1 - \frac{\theta}{360}\right) \delta_1 f^{(1-m)(2\delta_2-\delta_3)} \tag{22}$$

and

$$\alpha = \frac{\theta}{360} \alpha_1 f^{(2-m)\alpha_2-(1-m)\alpha_3} \tag{23}$$

Equations (22) and (23) show that Eq. (20) would be specific for the given cooker and product. To use it in another cooker would require a knowledge of f, θ and the product flow behavior index.

Heat transfer coefficient calculation

Heat transfer to a can is described by the equation:

$$m_c c_p \frac{dT}{dt} = UA\,(T_R - T) \tag{24}$$

where m_c is the mass of the can contents, c_p its specific heat, U the overall heat transfer, A the heat exchange area, T_R the retort temperature and T the bulk temperature. Integration of

Eq. (24) for a mean or constant c_p between t = 0, T = T_0 and t = t, T = T, results in:

$$\frac{m_c c_p}{A} \ln\left(\frac{T_R - T_o}{T_r - T}\right) = \bar{U}\, t \qquad (25)$$

where

$$\bar{U} = \frac{1}{t}\int_o^t U dt \qquad (26)$$

For thin walls the overall heat transfer coefficient is given by:

$$\frac{1}{U} = \frac{r_{in}}{r_{out}\, h_{out}} + \frac{r_{out} - r_{in}}{k} + \frac{1}{h} \qquad (27)$$

where r_{out}, r_{in}, h_{out} and h are external and internal can radii and heat transfer coefficients, respectively. For highly viscous materials and condensing steam as the heating medium, which is the case in this work

$$U = h \qquad (28)$$

for all practical purposes.

Equation (27) was used by Rao et al. (1985) and Rotstein et al. (1988) for cans in Steritort. From the standpoint of cooker design and analysis, it is useful to approach the problem considering the cooker as a heat exchanger. Then:

$$Q = w\, c_p\, (T_{out} - T_{in}) = h_m\, S\, (T_R - T)_m \qquad (29)$$

where w is material flowrate, $(T_R - T)_m$ the mean heat exchange difference, T_{out} exit temperature, T_{in} initial temperature, S material heat exchange area and h_m a mean heat transfer coefficient. For a cooker processing c cans per minute:

$$w = \rho\, V_{can} c \qquad (30)$$

$$S = c\, t\, A \qquad (31)$$

Using Eq. (30 and (31) in (29) leads to:

$$h_m = \frac{\rho\, V_{can}\, c_p}{A\, t}\, \frac{T_{out} - T_{in}}{(T_R - T)_m} \qquad (32)$$

where the value of h_m depends on the definition of $(T_R - T)_m$.

MATERIALS & METHODS

SEVERAL EXPERIMENTS were carried out on bench, pilot and commercial scale. On bench scale we simulated the stop-go mode of can rotation that occurs in commercial cookers. On a pilot cooker we determined the heat transfer coefficient of canned CSC and measured can rotation of tin-plated and tin-free lid cans. This rotation difference was also measured in commercial cookers under different conditions. In all cases we used either ETP or TFS cans. The latter have lids coated with polymeric lacquer. Can size was 300 × 407.

Cooker simulator

A positively driven can rotation assembly was designed to study the effect of different stop and go cycles, trying to reproduce conditions in actual rotary cookers. The simulator consisted of a can rotation assembly made of three stainless steel rollers driven by a variable speed motor (Fig. 2). The cans were placed in the cradle of two adjacent rollers. The drive motor turned the rollers, which in turn rotated the cans in contact. Any instantaneous can rpm, N, could be obtained by adjusting the drive motor speed. The motor was connected to a digital relay switch which turned the motor on and off in a cyclical manner to simulate the GO and STOP situations of cans in a cooker. The ON/OFF time could be varied on the relay switch to simulate different rotation angles, as well as different reel rpm. The roller assembly was installed inside a lab model FMC still retort. Test cans were fitted with standard Ecklund thermocouple fittings used in Steritorts. Thermocouple wires were connected to a data logger and a computer for automatic time-temperature data collection.

We simulated a cooker of D_r = 1.486 m. For a D = 3 in cans and N_r = 8 rpm this results, Eq. (2), in N° = 156 rpm. In the first set of tests, the instantaneous can rpm was varied for a constant cycle time t_1 = 7.5 sec (corresponding to a reel rotation N_r = 8 rpm) and a rotation time t_{GO} = 2.5 sec (corresponding to a rotation angle θ of 120°) resulting in a no-rotation time t_{STOP} = 5.0 sec. We changed N from 84 to 133 rpm, corresponding to changing f from 0.538 to 0.853. In the second set of experiments, N was kept constant at 132 rpm, t_1 = 7.5 sec and t_{GO} was varied between 1.43 and 2.50 sec. This corresponded to Eq. (4) (changing θ between 69° and 120°). Process conditions were as follows: Retort temperature of 132.2°C, cooling water temperature of 15.6°C, product initial temperature of 82.2°C and process time of 15.25 min. Product used was cream-style corn, with a viscosity of 32 ± 2 FMC Brabender units (Paddle B at 82.2°C) in 300 × 407 cans. Lethalities were calculated by the Ball formula method, taking into account both heating and cooling periods.

Pilot scale cooker

To obtain the heat transfer correlation based on Eq. (21), we used a Steritort pilot cooker (FMC Corp. Canning Machinery Div., San Jose, CA) installed at the pilot plant facility of The Pillsbury Company, Le Sueur, MN. All experiments involved cream style corn, in ETP or TFS 300 × 407 cans with a 42 pocket reel. Heating was done with steam, venting the Steritort until steam became visible. Heating time was 15 minutes, followed by a blow-down and cooling with a high pressure water spray. Ecklund copper-constant thermocouples were installed along the center line of each of six cans used per batch. The thermocouples were connected to a rotary contactor and lead wires transmitted the reading to a Toshiba 1100 portable computer via a Hanz-on Data logger (Hanz-on Data, Inc., Woodenville, WA). Retort temperature was set at 132.2°C and monitored throughout the process. The experiments were made spanning 5, 6.5, 8, and 10.6 reel rpm; 3.2 mm., 6.3 mm., and 11.1 mm head spaces; 6.5, 8.5 and 11 Adams consistencies, measured at 82.2°C. Actual values for K, M, ρ and c_p were measured as shown in Oudot et al. (1988).

The pilot unit simulated only the rotational motion of the commercial scale unit (see below) but not its spiral forward pattern. In another set of experiments, designed to compare can rotation in pilot and commercial cookers, the pilot unit was fit with a spiral that simulated the additional motion. ETP and TFS cans were processed and rotations counted using a mechanical counter (Rotstein et al., 1988, Rasmussen et al., 1989). All runs were at reel rpm N_r = 8 rpm, cooking time t = 15.25 min and retort temperature 132.2°C. The reel diameter was D_r = 1.283 mm.

Commercial cookers

We used Sterilmatic cookers (FMC Corp., Canning Machinery Div., San Jose, CA). In these machines cans enter the cooker and are indexed into a revolving reel; the cans move through the machine in a spiral pattern, rotating around their axis as they travel the lower section of each spiral. The cookers used were FMC commercial installations belonging to The Pillsbury Company (Tecumseh, Canada, Le Sueur, MN and Glencoe, MN) and Stokely USA (Cobb, WI). While all Pillsbury cookers had 42 pocket reels, Stokely USA cookers had a 47 pocket reel.

To understand the can material-cooker interaction, can rotation was measured under TFS conditioning or ETP burnishing. The first refers to a cooker were TFS cans have been processed for 100 hr. The second, to a cooker where ETP cans have been processed for at least 100 hr. A partially burnished condition was also experimented in Le Sueur. In a different experiment we verified the burnishing effect. Starting with a TFS conditioned cooker (Le Sueur cooker #1) we processed ETP cans up to 64 h, counting the accumulated rotations of ETP cans and TFS cans at 3.5 and 64 hr. The experimental conditions were 10.67 rpm reel frequency, 132.2°C retort temperature and 11.4 min processing time. The cans were 300 × 407 containing commercial CSC.

RESULTS & DISCUSSION

Can rotation and reel rotation

Figure 3 illustrates different combinations of f and θ values which result in n = 663 rotations. A decrease in n for given values of N° and t could be explained by a change in θ and/or a change in f. The resulting rotation performance would be very different, as illustrated in Table 1. The case considered

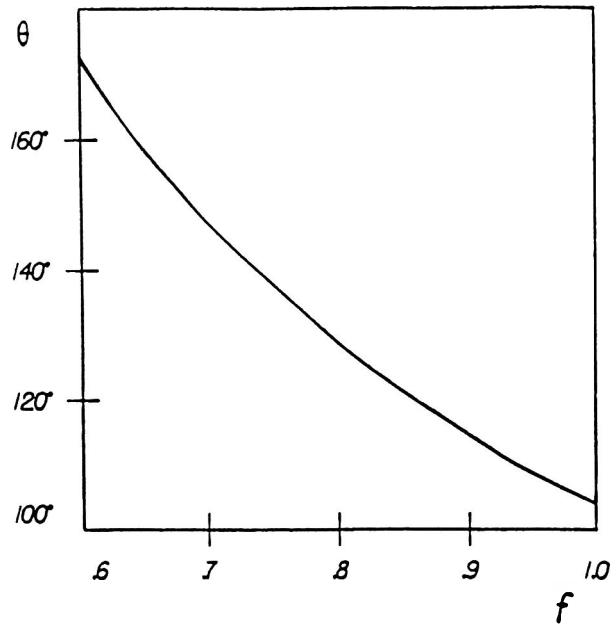

Fig. 3—f, θ combinations yielding the same n = 663 rotations.

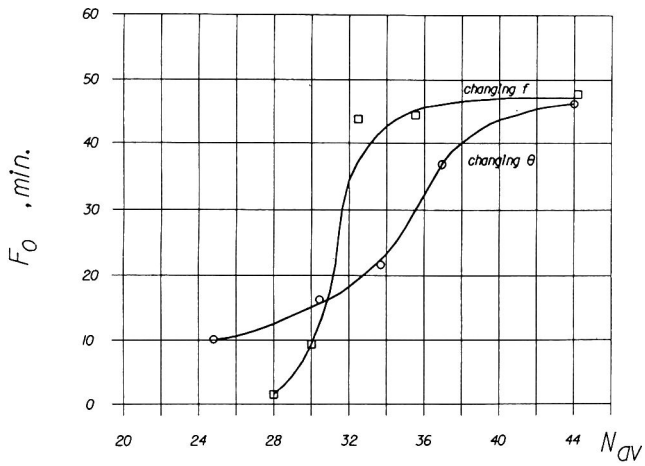

Fig. 4—Sterilizing value as a function of N_{av} for changing θ or changing f.

Table 1—Examples of alternative ways for a decrease in accumulated rotations from 775 to 411[a]

n	f	θ	t_{GO} sec/reel cycle	t_{rot}, min	N rpm	N_{av} rpm
775	1.00	120.0°	2.500	5.00	155	51.67
411	1.00	63.6°	1.325	2.65	155	27.67
411	0.53	120.0°	2.500	5.00	82	27.38
411	0.80	79.5°	1.656	3.33	124	27.38

[a] Basis N_r = 8 rpm, t = 15 min.

is one in which N_r = 8 rpm, D_r = 1.476m, D = 0.076m and t = 15 min. For f = 1, θ = 120° this results in n = 775 rotations. A decrease to n = 411 accumulated rotations was explored considering the three options shown: changing θ, changing f or changing both; t_{GO}, t_{rot} and N were calculated using Eq. (4), (7) and (8), respectively. There was a substantial difference between the three alternative possibilities, as indicated by the resulting t_{rot} and N, even if N_{av} was almost the same. At this stage we do not know which mechanism occurs, but we could explore the implications using the cooker simulator.

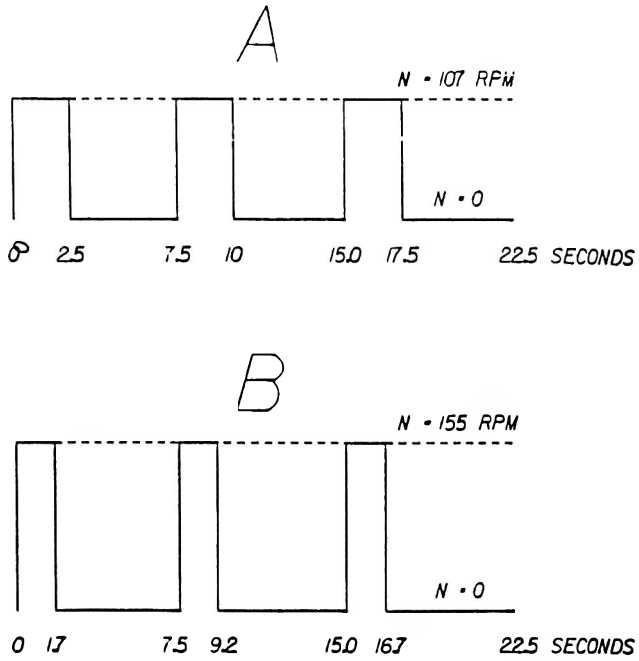

Fig. 5—Instantaneous can rpm as a function of time, for N_{av} = 35.6 rpm and N^c = 155 rpm. Case A: f = 0.69, θ = 120°. Case B: f = 1.0, θ = 82.7°.

Table 2—Change in rotation when scaling-up[a]

	ETP Cans				TFS Cans			
Equipment	n	s.d	%	fθ	n	s.d	%	fθ
Steritort	674	82	100	118.6	631	101	94	111.0
Steritort w/Spiral	570	37	85	100.3	563	20	84	99.1
Sterilmatic (LeSueur #1)	540	29	80	95.0	502	20	74	88.3
Sterilmatic (Tecumseh #1)	479	18	71	84.3	441	32	65	77.6

[a] Conditions: N_r = 8 rpm, t = 15.25 min, D_r = 50.5 in, T_R = 132.2°C, ETP burnished Sterilmatics, 42 pocket reel, 300 × 407 cans.

Cooker simulator studies

To further explore the issues, we simulated alternative rotation patterns in the cooker simulator (as indicated in M & M). The resulting sterilizing values for changing θ and changing f are shown in Fig. 4. Both cases showed an exponential decay, but it was more pronounced for changing f. Previous data showed that N° was not achieved (Rotstein et al., 1988), i.e. f<1. We doubted that there was only one possible value for θ, for different cans and cookers. Thus, it is reasonable to assume that in a real system we would have a combination of both cases. Notice that changing f implied changing the instantaneous can rotation for constant t_{GO}. On the other hand, if f=1 and θ decreased, we had a constant N which was active for less time. The differences are illustrated in Fig. 5.

Pilot and industrial scale experiments

Because can rotation decisively influences sterilization performance, it was important to determine how rotation in a pilot cooker compared with that in the corresponding full scale cooker. Table 2 shows results of accumulated rotations using ETP and TFS 300 × 407 cans. Figures refer to 15.25 min. processing time and percentage figures correspond to considering ETP cans in the Steritort as a base 100. The Sterilmatic runs were all made in ETP burnished cookers. There was a substantial decrease in rotations from pilot to full scale, for any can material. The difference in rotation between ETP and TFS cans was not substantial. We theorized this was due to the cookers being ETP burnished.

Table 3—Scaling-up with ETP Cans at N_r = 10.67 rpm[a]

Equipment	n	s.d	%	fθ
Steritort	699	24	100	92.2
Sterilmatic (LeSueur #1)	629	17	90	83.0
Sterilmatic (Tecumseh #1)	616	40	88	81.3

[a] Conditions: t = 11.4 min., T_R = 132.2°C, ETP burnished Sterilmatics, 300 × 407 cans.

Table 4—TFS and ETP 300 × 407 cans rotation, measured in commercial cookers[a]

Sterilmatic description	t_1 min	N_r rpm	ETP n	ETP s.d.	ETP fθ	TFS n	TFS s.d.	TFS fθ
Tecumseh #1[a], TFS Conditioned	14.6	8.7	622	33	105.1	505	65	85.3
LeSueur #1[b], Partially Burnished	11.4	10.67	585	5	103.2	516	26	91.1
LeSueur #3[c], Partially Burnished	11.4	10.67	608	19	107.3	576	8	101.6
Glencoe[d] TFS Conditioned	15.06	8.1	630	19	110.8	572	21	100.6
Cobb #2[e] TFS Conditioned	18.0	7.34	625	51	101.5	543	39	88.2
Cobb #3[f] TFS Conditioned	18.0	7.34	593	57	96.3	545	22	88.5

[a-f] Preheater and cooker temperatures: (a), (b) and (c): 111.1, 129.4°C; (d): 121.1, 121.1°C., (e): 126.7,126.7°C, (f): 128.9,128.9°C. Number of pockets per turn; a,b,c and d: 42; e and f: 47.

Table 5—Can rotation changes with ETP burnishing (LeSueur Cooker #1)[a]

Time	ETP Cans n	ETP Cans fθ	TFS Cans n	TFS Cans fθ
3.5 hr	585	103.2	517	91.2
64.0 hr	629	111.0	602	106.2

[a] 300 × 407 cans, t = 11.4 min, N_r = 10.67 rpm, preheater and cooker temperatures, 111.1 and 129.4°C respectively.

Table 6—Can rotation changes with ETP burnishing (LeSueur Cooker #3)[a]

Time	ETP Cans n	ETP Cans fθ	TFS Cans n	TFS Cans fθ
2.4 hr	607	107.1	576	101.6
70.0 hr	616	108.7	616	108.7

[a] 300 × 407 cans, t = 11.4 min, N_r = 10.67 rpm, preheater and cooker temperatures, 111.1 and 129.4°C respectively.

Note the fθ product which characterized the can-cooker interaction. This value was calculated using Eq. (9). It decreased for the same equipment when TFS cans were used instead of ETP cans. Moreover, it was significantly different in going from one Sterilmatic to another, even if both cookers were essentially the same in size and number of pockets.

To explore the influence of a change in reel rpm, some of the tests were repeated with ETP cans at N_r = 10.67 rpm. (Table 3). The decrease in rotation was substantial, although less steep than at 8 rpm. The characteristic product fθ also decreased in a less pronounced manner.

Rotation of different cans in commercial cookers

Table 4 shows results of rotation measurements of 300 × 407 ETP and TFS cans in different commercial cookers. Rotation decreased from ETP to TFS in all cases, the effect being very sensitive to the specific combination of conditioning, cooker and can material. The fθ product was different for all cookers and all cans. The two LeSueur cookers differed in the value of this interaction factor. Comparing the data in Table 4 with that in Table 3 for the LeSueur #1 Sterilmatic, the fully burnished cooker showed a larger fθ and hence more rotations than the same when partially burnished.

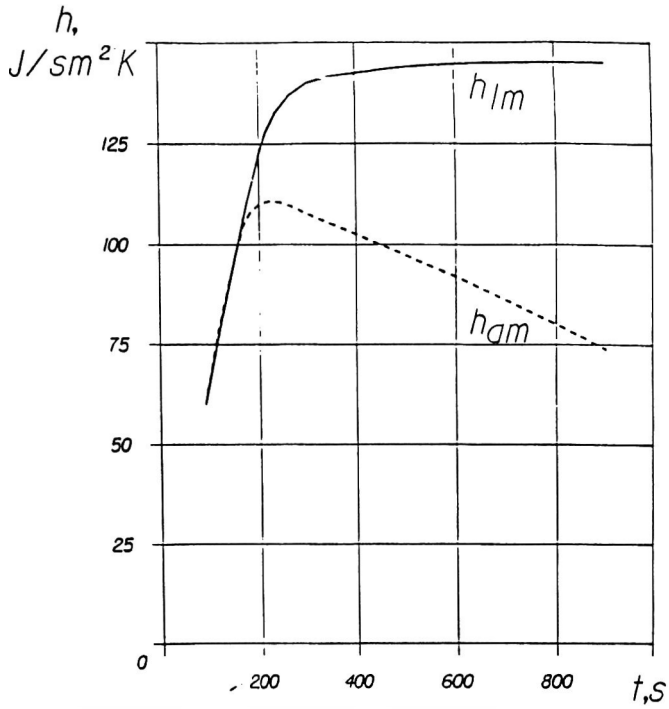

Fig. 6—Change in h_{lm} and h_{am} with residence time.

Time dependence of cooker conditioning

Table 5 and 6 show that the rotation performance of ETP cans improved, as accumulated time of processing the cans increased. The system behaved as if the cans changed the nature of the interaction surface, in a manner than increased rotation. We termed this effect of ETP cans on the cooker, burnishing. In the table, time zero is the time at which the cooker, after processing TFS cans for a long time, was switched to processing ETP cans. It follows from the data that both n and fθ improved, not only for ETP cans but also for TFS cans. It has been suggested that the ETP cans cleaned out any TFS deposit from the cooker shell and perhaps deposited small amounts of tin, which accumulated and acted as lubricants allowing improved performance.

Heat transfer

Pflug (1987) has indicated that it is important to consider the heat transfer process in a preservation problem. Equation (32) presents the question of which is the more adequate definition of h_m. Figure 6 shows representative results of change in h_m as a function of residence time. The arithmetic mean heat transfer coefficient, h_{am}, line corresponded to using the arithmetic mean:

$$(T_R - T)_{am} = \frac{T_R - T_{out} + T_R - T_{in}}{2} \qquad (33)$$

while the logarithmic mean heat transfer coefficient, corresponded to:

$$(T_R - T)_{lm} = \frac{(T_R - T_{in}) - (T_R - T_{out})}{\ln\left(\frac{T_R - T_{in}}{T_R - T_{out}}\right)} \qquad (34)$$

As it has been observed in tubular heat exchangers (Bird et al., 1960) h_{lm} tends to approach an asymptotic value as residence time increases. Conversely, h_{am} continued changing.

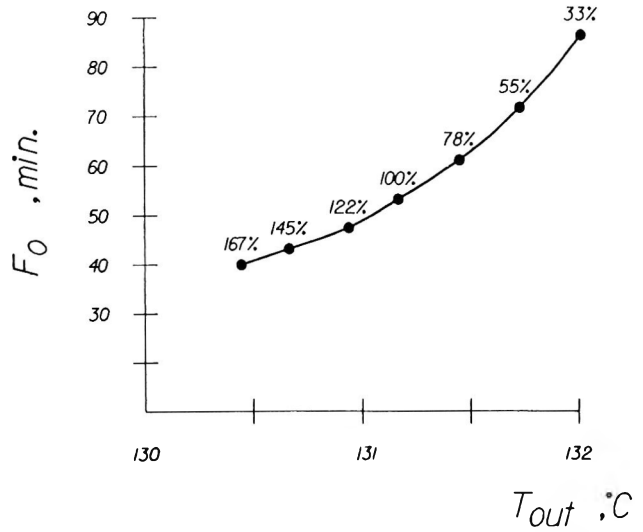

Fig. 7—F_o, T_{out} correspondence at changing consistencies. Relative consistencies are shown in graph as % figures. Basis 100% is K = 4.68 Pa sec^m. Case is a continuous 42 pocket cooker, t = 15 min, T_R = 132.2°C, N_r = 8 rpm.

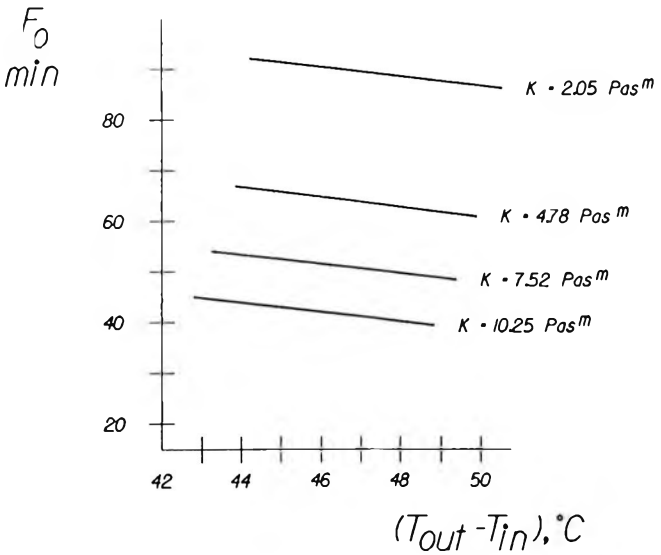

Fig. 8—Sterilizing value as a function of (T_{out} - T_{in}) in a 42 pocket cooker. T_R = 132.2°C, t = 15 min, consistencies are given at 85.6°C. T_{in} range is 82.2–88.9°C.

Figure 6 corresponds to CSC in a Steritort with N_r = 5 rpm, H = 11.1 mm. and 9.5 Adams viscosity. At lower agitation intensities the h_{lm} asymptote was delayed, while it was reached sooner at higher agitation intensity. It was thus convenient to use h_{lm}, bearing in mind that a minimum residence time was needed for the asymptote to be established. In that case, replacing Eq. (34) in Eq. (32) and using the net weight of can contents, h_{lm} is given by:

$$h_{lm} = \frac{m\, c_p}{A\, t} \ln \frac{T_R - T_{in}}{T_R - T_{out}} \qquad (35)$$

The head-space number can be calculated by measure or from:

$$\frac{H}{L} = 1 - \frac{4m_c}{\rho \pi D^2 L} \qquad (36)$$

The range of values of experimental variables resulted in Re between 4 and 268, Pr 414 - 12,339, Gr 27 - 9,414, Fr 0.02 - 0.09 and H/L 0.06 - 0.21. Starting from the model Eq. (31), several correlations were tested. The best fit corresponded to:

$$Nu = 0.492\, Re^{0.667}\, Pr^{0.37}\, (H/L)^{0.848} \qquad (37)$$

with r^2 = 0.977, indicating a negligible contribution from natural convection. Separate runs confirmed that the correlation did not improve using the Froude number.

Heat transfer and lethality

The predictive capability of Eq. (37) provided a tool to explore several issues. In particular, we were interested in determining evidence of existence of maximum or minimum lethalities and the process control implications of relationships between temperature, h_{lm} and F_o. To do this, we focused for simplicity on a process where heating was followed by instantaneous cooling, i.e., there is no added lethality from cooling. In that case (Rotstein et al., 1988):

$$F_o = \left[t - \frac{m_c c_p S}{h_{lm} A} \right] \exp \frac{\ln 10\, (T_R - 250)}{18} \qquad (38)$$

where

$$S = \sum_{i=1}^{I} (-1)^i \left[\frac{(T_R - T_o)(\ln 10)}{18} \right]^i \frac{(e^{-i h_{lm} A t / m_c c_p} - 1)}{i \times i!} \qquad (39)$$

and I is the number of summation terms needed to obtain convergence. By equating the partial derivatives $\partial F_o / \partial N_r$ and $\partial F_o / \partial H$ to zero, we found there was no maximum or minimum within the range of variables explored (5 - 10.6 reel rpm and 3.2 to 11.1 mm in head space). The N_r dependence is not trivial. Equation (37) shows that at increasing reel rpm the heat transfer coefficient will increase.

Because $F_o = F_o$ (h, t), at constant process time there was unequivocal correspondence between F_o and h. Equations (35) and (38) suggested, therefore, that there was correspondence between F_o and T_{in} and T_{out}, or F_o and T_{out} at constant T_{in}, when all other variables remained unchanged. Figure 7 illustrates the value of F_o corresponding to T_{out}, indicating the corresponding change in consistencies, when T_{in} was kept constant. This implied that, once the range of consistencies was defined, T_{out} could in principle be used to monitor F_o. Figure 8 shows how F_o varied with (T_{out} - T_{in}) for different consistencies. Another way of looking at the issue is given by Fig. 9. We have selected a base case (T_{in} = 82.2°C, T_R = 132.2°C, A = 0.0361m², c_p = 3,389 J/kg K, t = 15 min., N_r = 8 rpm, K_o = 0.0152 Pas^m, H/L = 0.14). This resulted in an upper value h = 248 J/sm²K, T_{out} = 146°C, F_o = 77.4 min. This is an asymptotic value and we could speculate that the same value could be approached by a product with different properties, resulting in a different h and F_o. Figure 9 was constructed assuming instantaneous cooling, using decreasing h values and obtaining the corresponding F_o with Eq. (38). If the process were such that we expected to obtain T_{out} = 131.67°C and the precision of our exit temperature reading is such that we can recognize a different T_{out} = 131.72°C, this would result in F_o = 62.9 min, corresponding to h = 216 J/sm²K. Using Eq. (37) we see that this could happen either because there was a 14.4% loss of rotation, or a 15% decrease in H/L or a 57.9% increase in consistency, all other variables being equal but the one indicated in each case. Of course, the further away from the asymptote, the less feasible it would become for T_{out} to misrepresent F_o.

CONCLUSIONS

TFS cans rotate less than ETP cans, the decrease depending on the length of time the TFS cans are used. The difference can be attributed to slippage with respect to ideal instantaneous

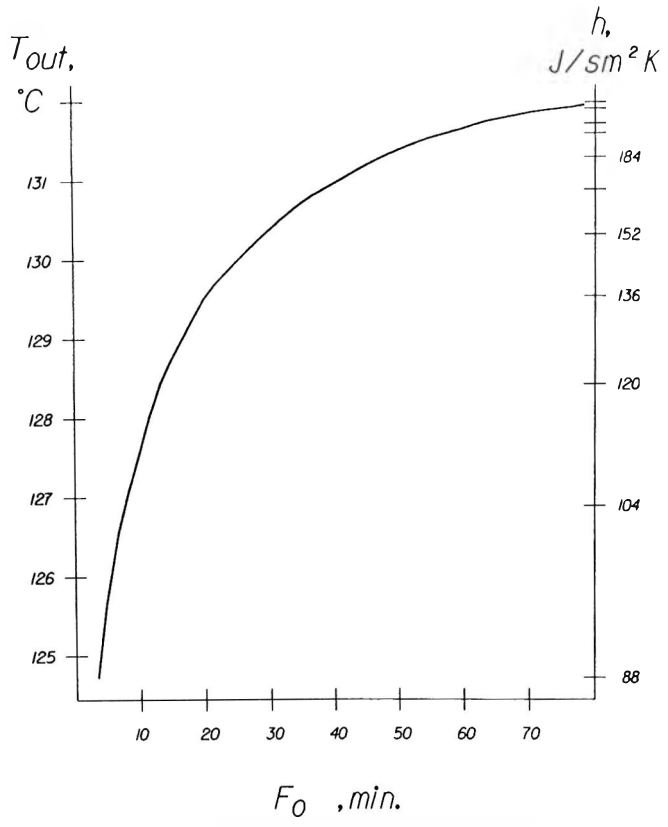

Fig. 9—Change in F_o when T_{out} changes due to a change in h. Base case, h = 248 J/msec²K.

rpm, and/or a change in angle spanning free rotation. In all cases the result is an exponential decay in F_o. The resulting potential danger makes it advisable to monitor can rotation when the situation warrants it. Whatever the can material, there may be a substantial decrease in can rotations from pilot to full scale cookers. Similar commercial cookers may also differ in can rotations. The rotation performance of cans in commercial cookers tends to improve with time. In TFS conditioned cookers, TFS cans always underperform ETP cans. In ETP burnished cookers, this was true, except in one case where TFS cans equaled ETP cans after 70 h of burnishing. The heat transfer coefficient depends on physical properties, headspace, can geometry and reel rotation. We could predict its value using Equation (37). The logarithmic mean heat transfer coefficient was a more suitable predictor than the arithmetic mean one. If physical properties could be kept within a narrow range of variability, we could use the product exit temperature as an indicator of accomplished F_o. Otherwise, we can only do that if we have ways to monitor physical properties on-line.

NOMENCLATURE

A	Heat exchange area
c	Cans per minute through cooker
c_p	Specific heat of food material
D	Can diameter
D_r	Reel diameter
Fo	Sterilizing value
$Fr' =$	$N^2 D/g$, Froude number based on can rotation
f	Coefficient accounting for non-ideal can rotation
$Gr' =$	$\rho^2 g D^3 \beta \Delta T_f / \eta^2$, Grashof number based on can rotation
$Gr =$	$\dfrac{\rho^2 D^{1+2m} \beta g \Delta T_o}{K^2 (N_r D_r)^{2m-2} \left(\frac{4\pi}{m}\right)^{2m-2}}$, Grashof number based on reel rotation
g	Gravitational acceleration
H	Head-space
h	Heat transfer coefficient
h_{am}	Arithmetic mean heat transfer coefficient
h_{fc}	Forced convective heat transfer coefficient
h_{lm}	Logarithmic mean heat transfer coefficient
h_{nc}	Natural convective heat transfer coefficient
h_m	Mean convective heat transfer coefficient
K	Consistency of food material
k	Thermal conductivity of food material
k_s	Constant characterizing can and product
L	Can height
m	Flow behavior index
m_c	Mass of can contents
N	Instantaneous can rotation frequency
N_{ow}	Average can rotation frequency
N_r	Reel rotation frequency
N^o	Ideal can rotation frequency (f = 1)
Nu =	h D/k, Nusselt number
$Nu_{nc} =$	h_{nc} D/k, natural convection Nusselt number
$Nu_{fc} =$	h_{fc} D/k, forced convection Nusselt number
n	Total can rotations during the process time.
$Pr' =$	$c_p \eta / k$, Prandtl number based on can rotation
$Pr =$	$\dfrac{c_p K (N_r D_r/D)^{m-1} \left(\frac{4\pi}{m}\right)^{m-1}}{k}$, Prandtl number based on reel rotation
$Re' =$	$D^2 N \rho / \eta$, Reynolds number based on can rotation
$Re =$	$\dfrac{(N_r D_r)^{2-m} D^m \rho}{k \left(\frac{4\pi}{m}\right)^{m-1}}$, Reynolds number based on reel rotation
r_{in}	Internal can radius
r_{out}	External can radius
S	Total material heat exchange area
T	Temperature
T_{in}	Can temperature entering the cooker
T_{out}	Can temperature leaving the cooker
T_R	Retort temperature
t	Processing time
t_1	Time for on full reel rotation
t_{GO}	Time, during a reel rotation, in which cans rotate
t_{rot}	Total can rotation time inside the cooker
t_{STOP}	Time, during a reel rotation, in which cans do not rotate
U	Overall heat transfer coefficient
V_{can}	Can volume
W	Material flow rate

Greek notation

β	Coefficient of volumetric expansion
η	Generalized viscosity of food material
θ	Angle describing the length of reel arc over which cans rotate.
ρ	Density of food material

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Membrane Reactor for Enzymic Deamidation of Food Proteins

J.S. HAMADA

ABSTRACT

A soy protein hydrolysate was deamidated with peptidoglutaminase (PGase) retained within a 30kd spiral membrane. Reactor was operated at 30°C and 6.5 L/m²/hr flux in recycle mode to 60% conversion, then in diafiltration mode for 2 hr. Time course, predicted by a Michaelis-Menten equation integrated for mixed zero- and first-order kinetics with the corrections for the ultrafiltration (UF) interactions, matched that measured experimentally. The equation could be used to predict the potential activity and performance of PGase in UF reactors to achieve control of reactions for the optimal enzymic process. At the end of the run, 96% conversion and 99% of PGase were obtained. This method has the potential of being scaled-up for the production of enzyme-free deamidated proteins.

Key Words: membrane-reactor, enzymes, deamidation, proteins

INTRODUCTION

PEPTIDOGLUTAMINASE (PGase) has been used in deamidation of casein and whey protein hydrolysates (Gill et al., 1985) and many food proteins and protein hydrolysates (Hamada, 1991a). Enzymic deamidation of food proteins improves solubility and other functional properties under mildly acidic (pH 4-6) conditions (Hamada and Marshall, 1989). Use of ultrafiltration (UF) in enzyme immobilization indicates the physical confinement or retention of enzymes in a defined region of space with retention of their catalytic functions (Cheetham, 1986). Because of the selective nature of the UF membrane this type reactor can be used for carrying out enzyme reactions using membranes that are impermeable to the enzyme but permeable to its substrates and products. Butterworth et al. (1970), Abbott et al. (1976) and Desslie and Cheryan (1981) were among the pioneers who developed continuous methods for enzymic hydrolysis or processing of starch, proteins and other molecules in a UF reactor. Chambers et al. (1976) reviewed the advantages of UF immobilization over conventional immobilization of enzymes. Dziezak (1990) emphasized the unlimited potential of using membranes in food biotechnology for enzyme extraction, purification and immobilization.

Hamada (1991b) employed a UF process to recover 97% of PGase after being reused 4 × in a 2-hr deamidation reaction at 30°C, using a 30-kd molecular weight cut-off (MWCO) membrane. The enzyme could be used at 25–30°C for 16 hr without loss of activity. The immobilization of PGase by containment was possible because of the relatively high molecular weight of PGase (and/or its dissociated subunits) and the enzyme stability. Chambers et al. (1976) reviewed the preparation, analysis, and performance of UF immobilized enzymes emphasizing the interaction of kinetics with UF effect. Cheetham (1986) also reported excellent reviews of enzyme kinetics and how they can be used in industrial biotechnology in general, and in membrane bioreactors in particular. The objective of the current investigation was to develop the kinetics for the large-scale deamidation of food proteins or protein hydrolysates with PGase immobilized in a 30-kd membrane.

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MATERIALS & METHODS

Preparation of PGase

B. circulans (ATCC #21590) cells were grown, harvested and extracted as previously described (Hamada et al. 1988). Cell extract, in 0.002M sodium phosphate buffer (pH 7.0), was ultrafiltered with a 30-kd spiral membrane (Amicon Corp., Lexington, MA) at 5°C in diafiltration mode at 10 mL/min flux. The retentate was freeze-dried and stored at −20°C until used.

PGase activity

Reaction mixtures containing 0.50 mg PGase and 10-20 micromoles amides from CBZ-L-Gln, t-BOC-L-Gln-L-Pro or Peptone type IV (Hamada, 1991b) were incubated at 30°C for 60 min and the ammonia content was determined as previously described (Hamada et al., 1988). The effects of buffer concentration and pH on PGase activity were studied. The progress of the PGase deamidation reaction of peptone IV in 0.05M phosphate buffer (pH 7.0) as a function of time was measured at 25 and 30°C.

Time course of peptone reaction

The PGase reaction time course using peptone type IV as a substrate was determined as previously described (Hamada, 1991a). The reaction was initiated by adding 50 mL solution containing 40 mg PGase to 850 mL peptone solution in 0.05M buffer (pH 7.0) containing 15.0g peptone. The reaction mixture was incubated at 30°C and 2 samples (10 mL each) were removed for ammonia analysis initially and every 30 min thereafter. Progress of the reaction was plotted as a function of time.

Reaction time course in UF recycle mode

A 900-mL solution containing 15g peptone, 40 mg PGase and 40 mmoles sodium phosphate buffer (pH 7.0) was ultrafiltered by the 30-kd membrane at 30°C for 4 hr in a recycling mode (Fig. 1) at a predetermined permeate flux rate of 6.5 L/m²/hr. The recycling mode was a concentration mode with both retentate and permeate recirculated to the reaction mixture reservoir. Samples from the reservoir were analyzed for ammonia content and progress of the deamidation

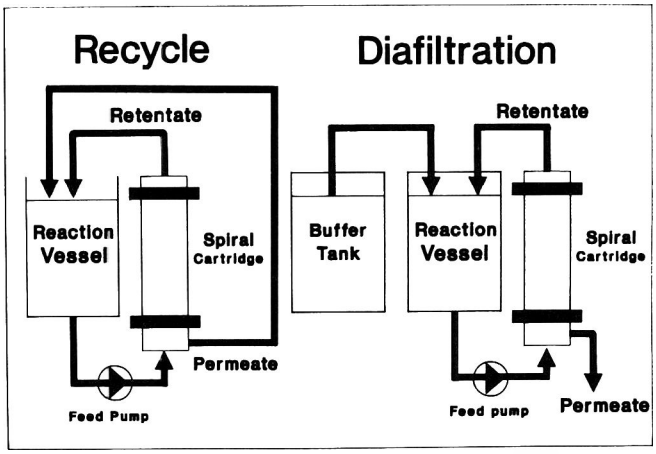

Fig. 1—Schematic diagram of the operational modes of a UF reactor system.

was calculated. Extent of deamidation at any point of the progress curve was expressed as the degree of conversion, X, which is $(S_0 - S)/S_0$, where S_0 is substrate concentration at 0 time and S, at t time. Releasing 0.40 mmoles ammonia from 1g peptone (on protein basis) was equivalent to 100% conversion or X value = 1.00 (Hamada, 1991b). The effect of UF on the reaction time course was expressed as an operational effectiveness factor (η_0), i.e., the ratio of amide conversion (at any point in the progress curve) to the corresponding point on the progress curve measured in the absence of the UF device.

Reaction time course in UF recycle and diafiltration modes

A solution (880 mL) consisting of 8g peptone and 24 mg PGase was placed in the 30-kd spiral membrane reactor, which was operated at 30°C at a flux rate of 6.5 L/m²/hr in recycle mode for 2 hr and diafiltration mode for 2 hr (Fig. 1). Samples were removed from the reaction vessel to monitor the peptone deamidation in both UF modes. Another operational effectiveness factor, η_0', was calculated for the second half of the curve as the conversion obtained with the UF module in diafiltration mode, divided by the conversion with the UF in recycle mode.

Maximizing conversion

The Michaelis-Menten equation integrated for mixed zero- and first-order kinetics (Hamada, 1991a) with effectiveness factors being incorporated into it (Eq. 5, Nomenclature) was used to calculate the amount of PGase required to complete the reaction at the end of the UF process. The reaction time course was determined as described, except for the amount of enzyme used (27.5 mg) and the starting time for the diafiltration (when X = 0.6), which was 105 min (calculated by Eq. 4, Nomenclature).

RESULTS & DISCUSSION

Parameters affecting PGase activity

The activity of an enzyme is determined by its concentration, the substrate concentration and availability, ionic strength, pH, and temperature. Enzyme kinetics, the study of these parameters and how they affect enzyme activity, provides an understanding of the reactions concerned and helps control to be developed. The specific activities of PGases I and II in 0.025 to 0.2M phosphate buffer, at pH 6–9 averaged 8 and 22 μmoles/mg/hr but reduced steadily to 4 and 16 μmoles/mg/hr at pH 4.0. The data were in agreement with original reports of Kikuchi and Sakaguchi (1973) who indicated that optimum pH of PGase I in Tris-HCl buffer was about 8.0 and PGase II had a broad pH profile of relatively high activity, about 5–11, with an optimum at 8.0. When soy peptone was used as substrate, results remained basically unchanged as the optimal pH range of PGase activity (20 μmoles/mg/hr) was 6–9. The activity reduced to 16 μmoles/mg/hr at pH 4. The optimal molarity of the phosphate buffer (pH 7.0) was 0.025–0.200 in which activity remained at 20 μmoles/mg/hr. As expected, peptone deamidation progressed faster at 30°C than at 25°C (Fig. 2). Activity of PGase, and hence the progress of peptone deamidation, tends to vary depending on composition and conditions. Thus in initial velocity determinations used in designing and optimizing a UF reactor, these parameters were kept constant.

Effect of recycling on PGase kinetics

The time course of peptone deamidation by PGase is presented in Fig. 3 (A). The progress of the reaction as a function of time was determined with the UF module in recycle mode at 6.5 L/m²/hr flux throughout the course of the reaction (B in Fig. 3). Comparing Fig. 3 B with A, the recycling apparently increased activity, which could have been due to better mixing of reactant and enzyme. The recycle bioreactor is usually operated under completely mixed conditions because of the wide range of distribution of residence times in the system (Cheryan, 1986). The effectiveness factor (η_0) averaged 1.04 for the first half and 1.15 for the second half of the reaction.

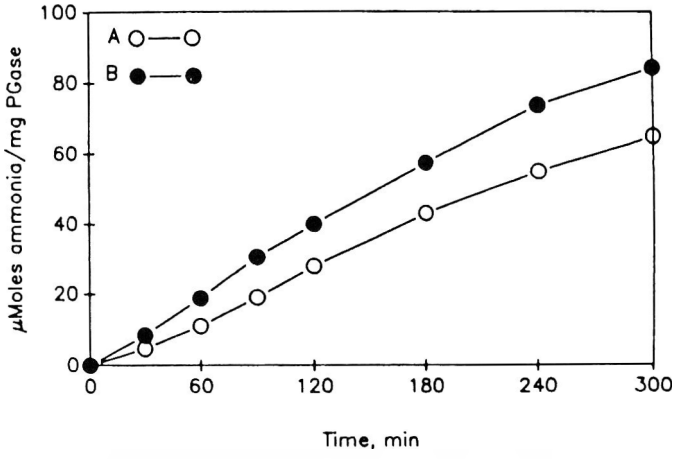

Fig. 2—Effect of reaction temperature on PGase deamidation of Peptone IV: (A) 25°C; (B) 30°C.

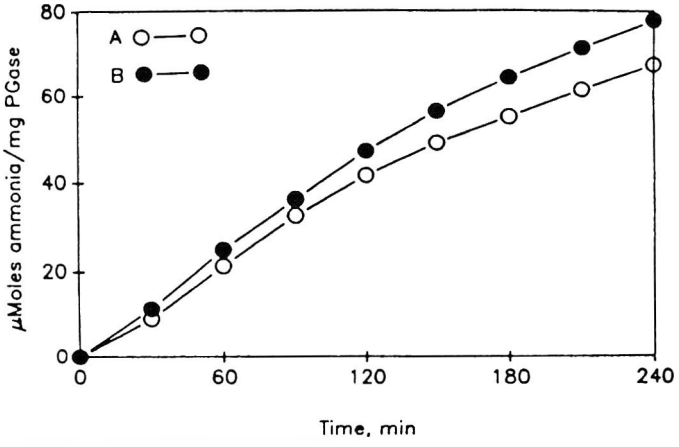

Fig. 3—Effect of ultrafiltration (UF) in recycling mode on PGase activity toward Peptone IV: (A) No transmembrane pressure applied, i.e. no UF; (B) Recycling at 6.5 L/m²/hr flux.

Hamada (1991a) previously used a Michaelis-Menten equation for mixed zero- and first-order reactions (Eq. 1, Nomenclature) to accurately predict the time course of PGase deamidation of the soy protein hydrolysate, which was measured experimentally (curve A shown in Fig. 3). K_m and V_{max} values of 1.67×10^{-4} M and 0.47 μmoles/min/mg were used to solve the equation. As suggested by Chambers et al. (1976) and Cheetham (1986), the effect of UF on enzyme kinetics could be described by Eq. (1), but with inclusion of the operational effectiveness factor (η_0) as in Eq. (2) and (3). Therefore, η_0 in the equations corrects for the increase in amide hydrolysis caused by the circulation of the reaction mixture. This must be used in predicting the time course of the reaction for a given substrate quantity (Eq. 2) and calculating the amount of enzyme required for its complete deamidation (Eq. 3).

Kinetics in recycle and diafiltration modes

The enzyme was recovered by switching from recycle to fractionation mode. At least 2 hr were required to completely separate the enzyme from the products in this reactor. Accordingly, the cut-off time for the switch was 2 hr. The diafiltration was also used to complete the reaction to the desired degree of conversion (X). In this reactor, the UF device was first operated at 30°C and 6.5 L/m²/hr flux in recycling mode for 2 hr, then in the diafiltration mode for 2 hr. The peptone

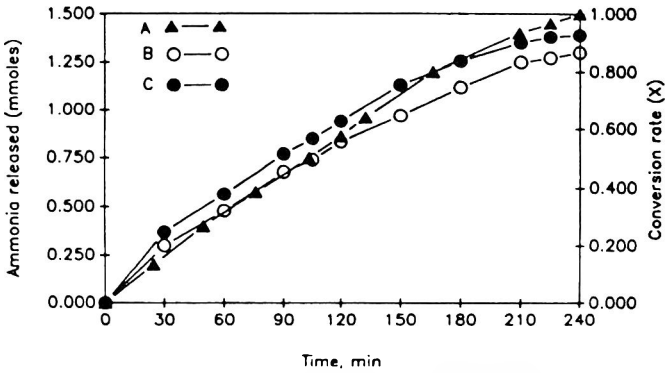

Fig. 4—Progress curves for Peptone IV deamidation by immobilized PGase in recycling mode (RM) and diafiltration mode (DM): (A) RM, E_0 = 22 mg; (B) RM for 120 min, DM for 120 min, E_0 = 22 mg; (C) RM for 105 min, DM for 135 min, E_0 = 26 mg.

substrate contained 1.5 mmoles amide which required 22 mg PGase, calculated by Eq. (3), to attain complete conversion in 4 hr. The time course of this reaction, with the module in two modes (B in Fig. 4) was compared to the predicted curve (A in Fig. 4) that was calculated by Eq. (2) while the module was only in recycle mode. The following is an example of solving Eq. (2) to calculate the time required to hydrolyze a certain number of amide groups, e.g., 0.5 mmol., by PGase:

V = 830 mL (containing 7.55 g peptone or 1.75 mmol amide).
X = 0.5/1.75 = 0.2857
S_0 = 2.1084 μmoles/mL
K_m = 0.167 μmoles/mL
V_{max} = 0.47 × 22.64 = 10.64 μmoles/min/mL
η_0 = 1.029 (from Fig. 3).

$$t = \frac{830\,[(2.1084 \times 0.2857) + (0.167 \times \ln 1.4)]}{10.64 \times 1.029}$$

$$= 49.9 \text{ min}$$

The activity of the enzyme in both curves (A and B) continued basically at the same rate until about 60% of the substrate was converted to product. The activity thereafter decreased during the course of reaction and the decline was more in B than in A. Accordingly, operating in a diafiltration mode reduced the rate of deamidation in the second half of the cycle (progress curve) to an average of 13% as compared to that in recycle mode. Thus, the operational effectiveness factor for this part of the cycle (η_o') was 0.87. At the end of this batch test a conversion of 0.85 was obtained (η_o' = 0.85). The decline in activity could have been due to the combined effects of the presence of the membrane and permeation. Therefore, the performance of immobilized PGase must be determined by including the interaction of the enzyme kinetics with such UF effects as suggested by Chambers et al. (1976) and Cheetham (1986). For our system, the interaction of PGase kinetics and UF and permeation effects could be easily described using Eq. (2) and (3) with incorporation of η_o', i.e., Eq. (4) and (5), respectively.

Maximizing PGase deamidation

As indicated earlier, a higher concentration of enzyme was needed to maintain the rate of reaction, so that completely converted substrates (X = 1.00) could be obtained in 4 hr. Industrially, the rate of reaction is usually controlled by varying the enzyme concentration and/or the period of reaction, in order to achieve the desired degree of conversion at a fixed reaction rate (Cheetham, 1986). The enzyme required for complete deamidation at the end of a UF test, 26 mg, was calculated by Eq. (5) with an η_o' value of 0.85. The cut-off time for switching from recycle to diafiltration, based upon X = 0.60, was 105 min, as calculated by Eq. (4). The reaction rate was slightly higher in those mixtures with greater amounts of enzyme (Fig. 4, C). The maximum conversion was, however, about 0.96 at the end of the process. Therefore, it is imperative to use a slightly higher concentration than that calculated by Eq. (5) (which should be regarded as the minimal level required). Fullbrook (1983) emphasized that the enzyme concentration calculated by this Michaelis-Menten expression should be regarded as the minimal level required, since it is unlikely that the enzyme would maintain its full activity during the entire course of the test.

Industrial application

An average of 99% of PGase was recovered after each run. The major advantage of membrane bioreactors over conventional batch enzyme catalysis is that the enzyme is retained within the system and can be reused many times, thus increasing productivity (Cheetham, 1986). Spiral membrane offers high product recovery because of the low adsorption of the membrane. Concentration polarization, the increase in the concentration of solute adjacent to the membrane, causes the filtration rate to decline logarithmically with increasing concentration of bulk solute. The control of concentration polarization is critical to the success of the bioreactor (Cheryan, 1986). Butterworth et al. (1970) reported concentration polarization problems, which resulted in severe fouling of the membrane. Use of the spiral-wound membrane apparently overcame this problem, since the reactor could be operated at 30°C for several hours without apparent loss in PGase activity. The use of YM hydrophilic spiral membrane could be an important factor contributing to the reduction of enzyme loss by adsorption or inactivation. The YM series of membranes (marketed by Amicon Corp., Lexington, MA) are more hydrophilic and have very little or no net charge, which may provide a practical solution to enzyme inactivation by adsorption, compared to PM polysulfone membranes (Cheryan, 1986). Furthermore, as noted by Dziezak (1990), spiral-wound membrane is the most popular flat membrane configuration for industrial separations. Its design induces turbulence of flow which keeps the membrane clean.

Using the UF module and Michaelis-Menten kinetics, both originally designed for industrial reactors, allows the possibility of scale-up of this UF laboratory method for larger scale production of enzymatically deamidated proteins. This technology would enable processors to work with large volumes, non-destructive conditions and minimal effect on the microenvironment (Dziezak, 1990). It also would lead to improved product quality, high energy efficiency and cost savings. Additionally, using the process would result in an enzyme-free product. This is important to the food processor since enzymes used in modifications are considered food additives (Cheryan, 1986).

CONCLUSIONS

A METHOD was developed for the PGase deamidation of a soy peptone hydrolysate, in which UF was applied in the recycling mode until 60% of the substrate was converted and then in a diafiltration mode until hydrolysis was complete. To correct for the influence of UF on progress of the deamidation reaction, an effectiveness factor was incorporated into the Michaelis-Menten kinetics equation integrated for mixed zero- and first-order rates. Thus the behavior of the reactor and the enzyme level required for complete deamidation could be predicted.

Nomenclature and Equations

$$t = V\{S_0 X + K_m \ln [1/(1-X)]\}/V_{max} \quad (1)$$

$$t = V\{S_0 X + K_m \ln [1/(1-X)]\}/V_{max}\,\eta_o \quad (2)$$

$$E_0 = V\{S_0 X + K_m \ln [1/(1-X)]\}/t\,V_{max}\,\eta_o \quad (3)$$

$$t = V\{S_0 X + K_m \ln [1/(1-X)]\}/V_{max}\,\eta_o\,\eta_{o'} \quad (4)$$

$$E_0 = V\{S_0 X + K_m \ln [1/(1-X)]\}/t\,V_{max}\,\eta_o\,\eta_{o'} \quad (5)$$

E_0 the enzyme quantity (in mg) present at zero time. $V_{max} = EK_2$, where EK_2 is the product of the enzyme present and its activity (v)
η_o the operational effectiveness factor for recycling
$\eta_{o'}$ the operational effectiveness factor for diafiltration
K_m Michaelis-Menten constant, expressed in μmoles/mL
S_0 the initial substrate concentration (μmoles/mL)
t time of reaction, min
v the initial rate of enzyme-catalyzed reaction (μmoles/min)
V the total volume of reaction mixture (mL) including the fluid in the circulation tube or the membrane
V_{max} maximum velocity of enzyme-catalyzed reaction, expressed in μmoles/min (per 1.0 mL) for Eq. (1), (2) and (4) and in μmoles/min/mg for Eq. (3) and (5)
X Conversion; $X = (S_0 - S)/S_0$

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Lactococcus Genus: A selective and Differential Agar Medium

N. AL-ZOREKY and W.E. SANDINE

ABSTRACT

An agar medium (Alsan), was selective for *Lactococcus lactis* subsp. *lactis* and *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*. The former developed white or yellow colonies; the latter gave blue-green or blue colonies. Eleven strains of *L. lactis* subsp. *cremoris* did not grow in this medium. The medium contained phenylethanol agar, lactose, glycine anhydride, lithium chloride and trimethoprim. In comparison with M17 medium, Alsan was not significantly different ($P > 0.05$) in recovering either bacterium. Furthermore, the medium inhibited the common food genera *Pseudomonas, Escherichia, Leuconostoc, Enterococcus* and *Lactobacillus*. Bacteriophage plaque sizes of 0.4–0.5 cm were demonstrated on the medium using a lactococcal bacteriophage.

INTRODUCTION

SPECIES of the *Lactococcus, Pediococcus* and *Leuconostoc* genera are important in the manufacture of fermented food products, including cheeses, buttermilk, yogurt, pickles and sausage (Tamime and Robinson, 1988; Tueber and Geis, 1981; Valerie, 1987). They are used for lactic acid production, pH lowering, the production of aroma compounds such as diacetyl, ripening of cheese and the production of substances which inhibit undesirable organisms (Al-Zoreky et al., 1991; Champagne et al., 1990; Daeschel, 1989; Daly et al., 1972; Elliker et al., 1964; Klaenhammer, 1988; Perdigon et al., 1990). These genera are nutritionally fastidous and require a well-buffered medium for good growth. The dairy mesophilic lactic acid bacteria (LAB) are recognized as *Lactococcus lactis* subsp. *Lactis* (LL), *L. lactis* subsp. *cremoris* (LC) and *L. lactis* subsp. *lactis* biovar. *diacetylactis* (LD) (Sandine, 1988, 1989). Several agar media are being used to grow these organisms (Kempler and McKay, 1980; Speck, 1984; Terzaghi and Sandine, 1975; Teuber and Geis, 1981; Thomas, 1973). However, a selective medium for their isolation has not been documented.

The objective of our research was to evaluate the efficiency of a new medium, Alsan, to recover *Lactococcus* spp, and to differentiate between those that utilize citrate and those negative for this trait.

MATERIALS & METHODS

Cultures and activation

Twenty-two strains from the *Lactococcus* genus were used (Table 1). Each culture was activated in M17 broth [Baltimore Biological Laboratory, Baltimore, MD] (BBL) at 30°C for 18–24 hr. Between transfers, strains were maintained in M17 agar deeps at 2–5°C.

Experimental medium and quantitative analysis

The newly developed medium (Alsan) contained the following ingredients per liter of distilled water: Phenylethanol agar (Difco), 35.5g; glycine anhydride (Sigma), 10g; lithium chloride (Sigma), 0.5g; sodium acetate anhydrous, 5g; potassium phosphate monobasic (J.T. Baker), 2g; ammonium citrate dibasic (J.T. Baker), 2g; arginine monohydrochloride (NB Corporation), 5g; bromocresol purple (Sigma), 0.02g; and α-lactose (Sigma), 5g. After autoclaving at 121°C for 15 min and tempering to 45°C in a water bath, 10 mL of 10% potassium ferricyanide solution and 10 mL of ferric citrate (FC) - sodium citrate (SC) solution (1g of FC + 1g of SC dissolved in 40 mL of distilled water) were added. The solutions were steamed for 30 min before being added to the tempered agar in order to detect citrate utilization (Kempler and McKay, 1980). Also, 5 mL of filter sterilized solution containing 10 mg trimethoprim (Sigma) was added to each L of medium. The final pH of the medium as required for citrate permease induction (Kempler and McKay, 1981; Reddy et al., 1971) was 5.9 ± 0.1. The other medium used for comparison was M17 (BBL) (Terzaghi and Sandine, 1975), a widely used medium for the cultivation of mesophilic LAB and their phages.

Each active bacterium was diluted in 0.1% sterile peptone water to give about 10^4 to 10^5 colony forming units (CFU) per mL before surface plating onto media using the Spiral Plater System (Model D; Spiral System). Inoculated plates were incubated at 30°C for 48 h in a Gas-Pak (BBL) anaerobic System. Colonies were counted using the designed counting grid and at least three replicates, each in duplicate, were counted on each sample. Statistical analysis was conducted using the analysis of variance, WormStat™ program from Small Business Computers, Amherst, NH.

Mixed culture study

Combinations of strains of LL, LD and LC were mixed in sterile peptone water to give 10^4 to 10^5 CFU/mL before plating (Spiral System) onto M17 agar and Alsan. Plates were incubated at 30°C for 48 hr in a Gas-Pak and then examined for numbers and colony appearance.

Screening bacteria for growth on Alsan

Several organisms (Table 2) were surface plated onto Alsan (Spiral Plater) after being activated in BHI broth or MRS broth and diluted in peptone water. Plates were incubated at 30°C or 37°C for 48 hr in

Table 1—Recovery of Lactococcus *spp. on Alsan selective agar as compared to M17 agar*[a]

Organism	Strain	Percent recovery (%)
Lactococcus lactis	ATCC 7962	99
	LM 2306	101
	C_2	0
	F_2D_2	100
	NCDO 497	96
	ATCC 11454	100
	ATCC 11955	96
Lactococcus diacetylactis	18-16	99
	26-2	99
	DRC 1	98
	222	101
Lactococcus cremoris	P_2	0
	BK 5	0
	107/6	0
	205	0
	KH	0
	163	0
	196	0
	189	0
	819	0
	203	0
	224	0

[a] Count in M17 agar was considered 100%.

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Table 2—Selectivity of Alsan agar as indicated by the presence or absence of growth of selected bacteria after incubation at 30°C or 37°C for 48 hr

Organism[a]	Growth on Alsan[d]
Streptococcus thermophilus CR5[b]	−
Leuconostoc cremoris J	−
Lactobacillus bulgaricus Y[b]	−
Lactobacillus isolate[b]	−
Pseudomonas aeruginosa ATCC 419	−
Escherichia coli V517	−
Listeria monocytogenes Scott A	−
Enterococcus faecalis CG 110	±[c]
Staphylococcus aureus	±
Salmonella typhimurium[b]	+

[a] *Each organism had about 4->6 CFU (log_{10}) per mL when surface plated (Spiral Plater) onto M17 or Tryptic Soy Agar.*
[b] *Incubation at 37°C for 48 hr.*
[c] *Only pinpoint colonies were found.*
[d] *+ = Present, − = absent.*

a Gas-Pak except plates of *Pseudomonas aeruginosa* which were incubated aerobically. Control media for this phase of the study were M17 agar and Tryptic Soy Agar (TSA).

RESULTS & DISCUSSION

Medium productivity and differential ability

Colonies of LL appeared white or yellow against a yellow background though strains LM2306 and NCD0497 further metabolized arginine with resulting higher pH (violet medium) due to ammonia production. On the other hand, LD grew as blue-green or blue colonies due to citrate metabolism (Fig. 1). Color of the medium was either yellow or violet or a combination. Both LL and LD grew in the medium without addition of lactose, producing a strong violet color due to arginine metabolism and ammonia liberation. Comparison between the CFU/mL recovered of LL and LD on M17 and Alsan is shown in Table 1. Statistical analysis revealed no significant difference ($P > 0.05$) between the two media. However, Alsan did not support the growth of LC strains (Table 1). An agar medium has been developed to differentiate LL and LC based on arginine metabolism (Thomas, 1973). Also, a differential broth was formulated by Reddy et al., (1971) to separate LL, LC and LD strains on the basis of citrate utilization and CO_2 production. Our further investigations revealed that LC 205 and KH strains were inhibited by lithium chloride present in the Alsan medium (unpublished data, Al-Zoreky and Sandine, 1991). This is an important finding, though studies of the sensitivity of dairy starters to other inhibitors and antibiotics have been reported (Orberg and Sandine, 1985; Reinbold and Reddy, 1974).

With one exception, all LL strains grew well on Alsan; strain C_2, however, was inhibited on the new medium (Table 1). Noteworthy in this regard was that Ribosomal RNA (rRNA) from strain C_2 also hybridized with 16S rRNA probes specific for LC (Salama et al., 1991). This indicated that LL C_2 was more closely related to LC than LL. All strains of LD grew as light blue or dark blue colonies due to citrate metabolism. A medium previously described by Kempler and McKay (1980) allowed differentiation between citrate positive and negative variants of LD. However, bacteria of other genera, such as *Leuconostoc* and *Lactobacillus*, are known for their utilization of citrate and production of diacetyl (Bednarski and Hammond, 1990; Bednarski et al., 1989; Kempler and McKay, 1980; Teuber and Geis, 1981). Citrate assimilation by such organisms can result in a false positive identification as LD on KM agar. Other media have been developed for LAB (Speck, 1984; Teuber and Geis, 1981), but none were found selective for the mesophilic group. Furthermore, it was found that Alsan medium without bromocresol purple, ferricyanide, FC and SC supported bacteriophage plaque sizes of 0.4 - 0.5 cm when LL ATCC 7962 phage was tested. Other media were found to result in smaller plaque sizes (Terzaghi and Sandine, 1975).

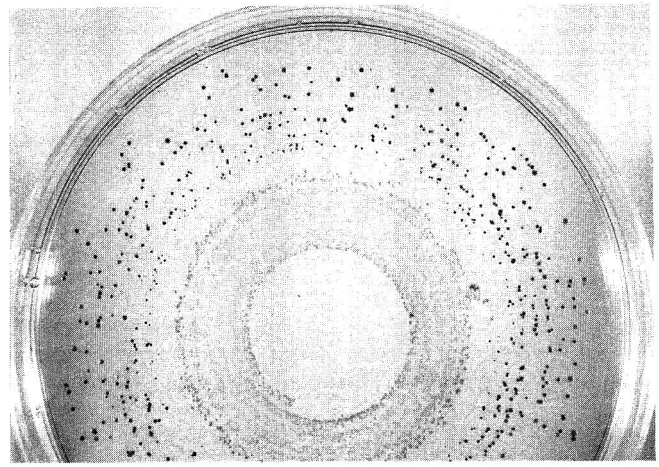

Fig. 1.–Appearance of colonies of Lactococcus lactis *ATCC 7962 (white to grey in color) center area and* Lactococcus diacetylactis *18–16 (light to dark blue in color) periphery on a spiral plate.*

Mixed cultures

After incubation, colonies from mixed cultures of LL, LD and LC developing on Alsan were of two types. The first was blue-green or blue in color indicating identity as LD. Yellow or white colonies were LL. In comparison with M17 plates, less dense growth was found on Alsan as a result of the inhibition of LC strains (unpublished data, Al-Zoreky and Sandine, 1991), and the count of M17 minus the Alsan count provided an estimation of the LC count. Consequently, both LL and LD would be selectively enumerated in the medium when present along with LC in a mixed or multiple strain starter or product derived therefrom. This may decrease cost and expedite the time required to isolate pure cultures from strain mixtures. Furthermore, the absence of growth of LC on Alsan emphasizes additional differences that exist between group N lactococci to those known already (Reddy et al., 1971; Speck, 1984; Teuber and Geis, 1981; Thomas, 1973).

Selection effectiveness of Alsan

Results indicated that Alsan completely inhibited *Leuconostoc, Streptococcus thermophilus,* and *Lactobacilli* (Table 2). Such LAB may be present in cheeses and fermented milk along with LL, LC and LD. Consequently, Alsan can selectively allow growth of LL and LD to obtain pure cultures. In contrast, M17 which is not selective, allows the growth of all organisms tested. While *Escherichia coli, Pseudomonas aeruginosa*, and *Listeria monocytogenes* were completely inhibited by Alsan, *Enterococcus faecalis* and *Staphylococcus aureus* grew poorly and produced only pinpoint colonies which were hard to count. Interestingly, *Salmonella typhimurium* grew on the medium, though Alsan contained phenylethanol which is inhibitory to gram negative bacteria.

The newly developed medium will be helpful in monitoring the growth of group N lactococci, especially when co-cultured with certain food spoilage and pathogenic organisms for evaluating their potency to inhibit such undesirable microorganisms. In this regard, it is well known that the inhibition of harmful organisms is dependent on the number of certain LAB present, as reported by Champagne et al., (1990), Schaack and Marth (1988) and Elliker et al., (1964).

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Ultrafiltration for Recovery and Reuse of Peptidoglutaminase in Protein Deamidation

J.S. HAMADA

ABSTRACT

Ultrafiltration (UF) of *B. circulans* cell extract using a 100kd hollow-fiber membrane had no effect on peptidoglutaminase (PGase) activity and allowed 34 and 26% of PGases I and II to permeate. UF, with 30kd spiral membrane, slightly increased PGase activity in retentate but no activity was detected in permeate. Retentate was used to sequentially deamidate four batches of a soy protein hydrolysate at 30°C for 2 hr, then PGase was recovered by UF. The 100kd membrane resulted in substantial PGase loss in sequential tests due to permeation and inactivation. A 100% conversion and 97% of PGase were obtained after tests with the 30 kd membrane. Multiple recovery and use of PGase by a suitable membrane is possible.

INTRODUCTION

IMMOBILIZATION of biocatalysts by attachment to solid surfaces, or entrapping them in a confined region with membranes, allows more efficient continuous processes with reuse of biocatalysts (Cheryan, 1986b). This can lower enzyme costs, improve process control, and the effective use of enzymes in food processing. Ultrafiltration (UF), an approach different from the conventional conception of immobilization, has been used as a means of recovering and reusing enzymes in continuous reactors (Pitcher, 1980). Blatt et al. (1968) reported the potential of using UF membranes for separation of higher-molecular-weight biologically active substances from lower-molecular-weight solutes. Butterworth et al. (1970), Abbott et al. (1976), and Desslie and Cheryan (1981) were among pioneers who developed continuous methods for enzymatic hydrolysis or processing of starch, proteins and other molecules in a UF reactor. Note also that UF processing is gaining application in the food industry due to advances in membrane technology (Modler and Jones, 1987). Dziezak (1990) stressed the unlimited potential of using membranes in food biotechnology for enzyme extraction, purification and immobilization.

Peptidoglutaminase (PGase) produced by *Bacillus circulans* has recently been used in the deamidation of casein and whey protein hydrolysates (Gill et al., 1985) and many food proteins and hydrolysates (Hamada et al., 1988; Hamada, 1991a). PGase deamidation of soy protein improved solubility and emulsifying properties under mildly acidic conditions (Hamada and Marshall, 1989). In decomposing high-molecular-weight protein substrates for PGase deamidation, reducing protein size is an essential step. Hamada and Marshall (1988) reported a quantitative relationship between the extent of deamidation and the degree of protein hydrolysis combined with heat treatment. Proteolysis will produce polypeptides many times smaller than PGase. Kikuchi and Sakaguchi (1973) reported the molecular weight (MW) of PGase I, hydrolyzing C-terminal Gln, and PGase II, hydrolyzing all other Gln, to be 200 kd in a non-dissociating salt and pH environment and 90 kd and 125 kd, respectively, in dissociating conditions of 0.1M KCl and pH 10.9.

Accordingly, a UF membrane with a suitable pore size, e.g., 100 kd MW cut-off (MWCO), should be impermeable to undissociated PGase but permeable to food proteins and deamidated products. Thus, employing UF in PGase recovery for recycling would allow the production of enzymatically deamidated food proteins with enhanced solubility and functional properties. The objectives of the current investigation were to study the effect of UF on the specific activity of a *B. circulans* cell extract and determine whether UF membrane technology could be used to separate PGase from deamidated peptides and provide means for multiple reuse of the enzyme in continuous reactors.

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MATERIALS & METHODS

Materials

B. circulans (ATCC #21590) was obtained from the American Type Culture Collection, Rockville, MD. Peptone type IV and CBZ-L-glutamine were purchased from Sigma Chemical Co., St. Louis, MO. t-BOC-L-glutaminyl-L-proline was purchased from Peptides International, Louisville, KY. The BCA (bicinchoninic acid) protein assay reagent was purchased from Pierce Chemical Co., Rockford, IL. Other chemicals were reagent grade of the highest purity obtainable.

Preparation and assay of PGase

B. circulans cells were grown and harvested and then used to prepare PGase cell extract as reported (Hamada et al., 1988). The cell extract and its UF fractions were evaluated for PGase I and II activities using the synthetic substrates CBZ-L-glutamine and t-BOC-L-glutaminyl-L-proline, respectively. The deamidating ability of PGase preparations towards a commercial soy protein hydrolysate was assessed using peptone type IV as substrate. To 5 mL substrate containing 25–50 μmoles amides in 0.05M phosphate buffer (pH 7.0), 1 mL enzyme preparation containing 0.5–1.0 mg protein was added and the reaction mixtures were incubated at 30°C for 2 hr. The ammonia content of the samples was determined with a model 95–10 ammonia electrode (Orion Research Inc., Cambridge, MA). One unit of enzyme activity releases 1μm ammonia from substrate after 2 hr incubation at 30°C.

PGase purification by ultrafiltration

A hollow-fiber membrane (H1P100-43), with MWCO of 100 kd and a spiral-wound S1-Y30 membrane with MWCO of 30 kd, both from Amicon Corp (Lexington, MA), were used to purify 400 mL of cell extract containing 0.05% protein. Membranes were mounted to a CH2 Amicon system. Based on the manufacturer's recommendation, the 100 kd membrane was first coated with ovalbumin prior to use to prevent protein binding, but the spiral membrane (purchased while UF experiments were in progress,) was not. The UF unit was operated at 5°C in the diafiltration mode, (see Fig. 1, Hamada, 1991 a),in which the ultrafiltrate was replaced by the continuous addition of 0.01M phosphate buffer (pH 7.0) from a separate reservoir, for 2 hr at an average flux of 20.0 L/m^2/hr for the 100 kd membrane and 6.5 L/m^2/hr for the 30 kd membrane (3 buffer volumes). Specific activities of PGase in the retentate and permeate fractions were measured.

UF recovery for multiple reuse of PGase

The 100 kd MWCO or the 30 kd MWCO membrane was used to ultrafilter about 1500 mL of 5% peptone solution prior to its use in PGase deamidation. This was to avoid contamination of enzyme with relatively high-MW peptones rejected by the membrane. A 1% peptone solution (750 mL in 0.05M phosphate buffer, pH 7.0), made of the prefiltered peptone, was deamidated by incubation with 50–60 mg PGase (obtained from retentate) for 2 hr at 30°C. The enzyme corresponded to an initial E/S ratio 1.5 × higher than that required for complete peptone deamidation. The enzyme was separated from the

Table 1—Effect of ultrafiltration on activity and yield of peptidoglutaminase (PGase) using a 100 kd MWCO membrane

		CBZ-Gln[a]		BOC-Gln-Pro[b]		Peptone IV	
	Protein (mg)	Total units (× 10^2)	Specific activity (units/mg)	Total units (× 10^2)	Specific activity (units/mg)	Total units (× 10^2)	Specific activity (units/mg)
Cell extract (feed)	186	47.4	25.5	52.6	28.3	51.2	27.4
Permeate	67	15.2	22.5	13.9	21.3	13.8	20.6
Retentate	119	26.4	22.3	36.4	30.6	36.9	31.0

[a] N-Carbobenzoxy-L-glutamine used to assay PGase I.
[b] N-tert-Butoxy-carbonyl-L-glutaminyl-L-proline used to assay PGase II.

Table 2—Effect on ultrafiltration on activity and yield of peptidoglutaminase (PGase) using a 30 kd MWCO membrane

		CBZ-Gln[a]		BOC-Gln-Pro[b]		Peptone IV	
	Protein (mg)	Total units (× 10^2)	Specific activity (units/mg)	Total units (× 10^2)	Specific activity (units/mg)	Total units (× 10^2)	Specific activity (units/mg)
Cell extract (feed)	196	23.3	12.0	66.5	33.9	63.5	32.4
Permeate	40	0	0	0	0	0	0
Retentate	155	22.7	14.6	66.1	42.7	64.4	41.6

[a] N-Carbobenzoxy-L-glutamine used to assay PGase I.
[b] N-tert-Butoxy-carbonyl-L-glutaminyl-L-proline used to assay PGase II.

Table 3—Enzyme recovery and extent of peptone deamidation upon reuse of PGase retained by PM-100 membrane

Sequential test	Activity (Units × 10^2)						Peptone deamidation	
	PGase I			PGase II				
	Feed	Retentate	Permeate	Feed	Retentate	Permeate	g used	%
1	13.5	10.2	3.3	18.1	13.6	4.0	7.5	59.0
2	9.5	6.9	2.3	12.7	9.2	2.7	7.0	57.8
3	6.4	3.8	1.5	8.6	4.9	1.7	6.5	32.0
4	3.5	1.2	0.9	4.5	1.1	1.0	6.0	25.0

deamidated peptone by UF using a 100 kd or 30 kd membrane at 20.0 or 6.5 L/m^2/hr flux, respectively, in diafiltration mode for 1 hr, followed by concentration mode for 1 hr at 25°C. At the end of each process, 1.2L of deamidated peptone and 150 mL retentate were obtained. This process of deamidation and UF separation of PGase was sequentially repeated three more times with volumes and peptone quantities adjusted for residual enzyme after sampling. Specific activities of retentate and permeate were determined before and after each test. Peptone deamidation was determined by measuring the ammonia released enzymatically in the permeated peptone after each test. The extent of deamidation was calculated as the ratio of ammonia released enzymatically to either total amides in the peptone (% deamidation) or the fraction of peptone amides that could be deamidated by PGase (conversion ratio).

PGase immobilization by gel adsorption

Calcium phosphate gel was loaded by stirring 15g gel with 100 mL dialyzed cell extract containing 60 mg protein in a 0.005M phosphate buffer (pH 7.0), at 600 rpm mixing and 4°C for 1 hr. The immobilized enzyme was added to 200 mL of 1% peptone type IV in 0.025–0.085M phosphate buffer (pH 6.0–7.5) and the mixture was stirred at 30°C for 30 min, then centrifuged. The ammonia in the supernatant was measured and the conversion of carboxyamide to carboxyl residues was calculated. The gel was used 5 more times to deamidate peptone type IV and the residual activity was measured each time. In one experiment, peptone solutions, before use as a substrate, were dialyzed overnight at 4°C against water using a cellophane membrane with molecular weight cut-off of 1 kd.

Chemical analyses

Protein content of the soy protein hydrolysate was measured by the Kjeldahl method (AOAC, 1980). Protein content of the PGase preparations was determined by the BCA method of Smith *et al.* (1985). The amide content of the soy protein hydrolysate was measured according to Wilcox (1967).

RESULTS & DISCUSSION

Partial purification of PGase by ultrafiltration

UF using a 100 kd MWCO membrane to fractionate *Bacillus circulans* cell extract had no effect on the activity of PGase rejected by the membrane (Table 1). Substantial amounts of PGase were lost in this process as 34% of the units of PGase I and 26% of II were lost to permeation through the membrane. The permeation of PGase could be due to the broad pore size distribution of the membrane. PGase had a nominal average molecular weight of 200 kd and could be dissociated into 90 kd and 125 kd species, depending on pH and salt conditions as indicated by Kikuchi and Sakaguchi (1973). The broad pore size distribution of the membrane (Cheryan, 1986a) may have accounted for the leakage that occurred through the 100 kd MWCO membrane. This hollow-fiber P-100 membrane was coated prior to its use in order to form a secondary membrane of protein (Mazid, 1988), which should prevent binding and may increase the percentage rejection of the enzyme by the membrane. There was no indication that this approach altered the effective pore size of the initial membrane in a manner that might have reduced leakage. Since the loss of both PGases I and II activities in the ultrafiltrate was substantial, the use of a membrane with MWCO of less than 100 kd should be tested to overcome this problem.

Table 2 presents the specific activity of PGase in the cell extract and its two UF fractions, using a spiral membrane with a MWCO of 30 kd. The specific activity of the starting cell extract for this membrane was different from that of the cell extract used with the high MWCO (100 kd) membrane (Table 1). This was probably due to preparation conditions and/or inactivation by freezing and thawing upon multiple use of the enzyme. UF with a 30 kd MWCO membrane increased the specific activity of PGase (I and II) in retentate by 1.25 fold, with 99% of the activity being recovered in the retentate. No activity was detected in the permeate fraction (Table 1). Apparently, the nominal average molecular weight of PGase was greater than 30 kd, as no activity was lost to permeation through the membrane.

Peptone deamidation and PGase recovery by 100 kd membrane

The 100 kd MWCO membrane was used for separation of PGase from reaction products in the deamidation of the pep-

Table 4—Enzyme recovery and extent of peptone deamidation upon reuse of PGase retained by S1-Y30 membrane

Sequential test	Activity (Units × 10^2)				Peptone deamidation	
	PGase I[a]		PGase II[a]			
	Feed	Retentate	Feed	Retentate	g used	%
1	6.5	6.5	19.6	19.4	7.5	58.6
2	6.0	5.9	18.1	17.9	7.0	58.9
3	5.5	5.4	16.6	16.4	6.5	58.2
4	5.0	4.8	15.2	14.9	6.0	59.2

[a] No PGase activity was detected in the permeate.

tone type IV to determine whether enzyme permeation would diminish with continuation of the UF process. The retentate was used 4 × to deamidate four batches of the peptone. Each test consisted of two steps, the first was carried out by incubating PGase mixed with peptone at initial E/S ratio that was 1.5 × the amount sufficient for complete deamidation in 2 hr at 30°C. The second step was a UF test that was carried out for removal of enzyme from the deamidated peptone. The rate of amide conversion in the peptone, as well as the PGase activity in feed, retentate and permeate for each batch run, are reported in Table 3.

When reaction mixtures were subjected to sequential UF tests to recover the enzyme, there was a substantial loss of PGase units through permeation and inactivation. The percentages of initial enzyme activity in the permeate solutions were 25, 24, 24 and 25 for PGase I and 22, 21, 20 and 22 for PGase II. Thus, an average of 24 and 21% of the total activity units of PGases I and II, respectively, in the feed permeated through the membrane and were lost after each run. Also, there was considerable reduction in the activity of PGases in the retentates recovered after the 3rd and 4th tests. The percentages of initial enzyme activity in retentate solutions varied from one test to another and were 76, 72, 59 and 35 for PGase I and 75, 73, 59 and 25 for PGase II, respectively, in the sequential tests. The addition of permeation percentage and retention percentage was considerably less than 100% for the third and the fourth tests. This was an indication of enzyme inactivation during batch processes, which apparently started after the 1st test and continued into the fourth test. Specific activites were 22, 18, 16, and 7 for PGase I and 30, 22, 14, and 7 for PGase II in the permeates collected after each batch test. Due to both permeation and inactivation, 11 and 8% of starting units of PGases I and II were only recovered in the final retentate.

Enzyme loss during sequential tests was probably due to the combined effects of broad pore size distribution of membrane (Cheryan, 1986a), decomposition by shear forces (Kennedy and White, 1986) and the chemical nature of the base polymer of the membrane, which could have caused substantial adsorption (Cheryan, 1986a; Mazid, 1988). Apparently pretreating the fibers with albumin did not overcome adsorptive losses and the protein coating of the P-100 membrane was ineffective in that regard. Protein binding and fouling by PM (polysulfone) membranes can cause severe enzyme inactivation (Cheryan, 1986a), even after protein coating of the membrane (Mazid, 1988). Such a secondary membrane should not attract further adsorption, but in reality, however, the protein binding process could prevail indefinitely due to random protein denaturation (Mazid, 1988).

The extent of peptone deamidation after each test is indicated in Table 3. The initial volume of the peptone solution was reestablished before each batch test to account for reduction in the amount of enzyme available due to sampling. The conversion of amide groups to carboxyl groups in peptone IV, considering that an average of 58.6% of the amides of the peptone was the maximum amount of PGase deamidation, was 1.0 for the first and second tests, and 0.6 and 0.5 for the third and fourth tests, respectively. Those levels of conversions corresponded to 59, 58, 32, and 25% deamidation of the peptone. The initial E/S ratio was sufficient to complete the peptone deamidation and the amount of peptone used in each test was dependent on the residual enzyme after sampling. Hence the decline in conversion was due to the drop in the enzyme-to-substrate ratio in the third and the fourth tests. As discussed, the decreasing effectiveness of PGase was due to activity losses by both permeation and inactivation. UF may still be a suitable technique for recovering substantial PGase enzyme after being used in the deamidation reaction. That is, providing the loss of protein and enzyme activity can be overcome by using a suitable membrane. A membrane with a MWCO of about 30 kd–50 kd of more hydrophilic material, may be suitable to ensure no loss of activity as deamidated soy protein hydrolysate is removed in the ultrafiltrate.

Peptone deamidation and PGase recovery by 30 kd membrane

The 30 kd membrane was used to filter the protein hydrolysate solution to remove species with molecular weights > 30 kd MWCO in order to achieve complete separation of peptone from enzyme at the end of a UF test. The permeate fraction contained 99.5% of the peptone protein. The retentate, from PGase purification by the 30 kd MWCO membrane, was used to deamidate 4 batches of the peptone. As in the 100 kd MWCO membrane tests runs, an experiment was designed so that the first peptone batch was completely deamidated before the enzyme was recovered by the UF step. The amide conversion in the peptone was 1.0 and no PGase I or II permeated through the membrane or was lost to inactivation after each test as evidenced by the unchanged specific activities of 14.5 and 42.7 units/mg for PGase I and PGase II (Table 4).

The high level of recovery of PGase, (97% after the 4th test), demonstrated that the membrane was effective in eliminating the loss of PGase activity due to permeation or inactivation. Both the MWCO and the material of the membrane may have been responsible for the complete recovery and successful reuse of PGase in this series of sequential batch processes. The use of YM hydrophilic membrane, instead of the PM polysulfone membrane, could have been an important factor contributing to elimination of enzyme loss by adsorption and inactivation. The YM series of membranes (marketed by Amicon) are more hydrophilic and have very little or no net charge. This may provide a practical solution to enzyme inactivation by adsorption, compared to PM membranes (Cheryan, 1986a). UF with the S1-Y30 membrane may be a very suitable technique for recovering most of PGase enzyme after being used several times in the deamidation reaction of food proteins or protein hydrolysates with a nominal average MW of less than 30 kd.

Gel adsorption

PGase could be physically adsorbed upon calcium phosphate gel with no loss of activity. However, when the immobilized PGase was used to deamidate soy peptone by stirring the loaded gel and peptone mixture at 30°C for 30 min, 87% of PGase activity was lost under multiple use in 6 sequential batch tests (Fig. 1). Treatments such as lowering ionic strength and pH did not improve the adsorption of PGase on the gel (Fig. 1). The data suggest that calcium phosphate gel was an inappropriate support for adsorption immobilization of PGase, in spite of the possibility of recovering and reusing desorbed enzyme.

CONCLUSIONS

IMMOBILIZATION of PGase by containment in a UF membrane was possible taking advantage of the relatively high MW of PGase and enzyme stability. However, proper choice of membrane pore size and/or membrane material were critical to any practical application. PGase was retained within a 30 kd MWCO spiral membrane and could be reused many times at

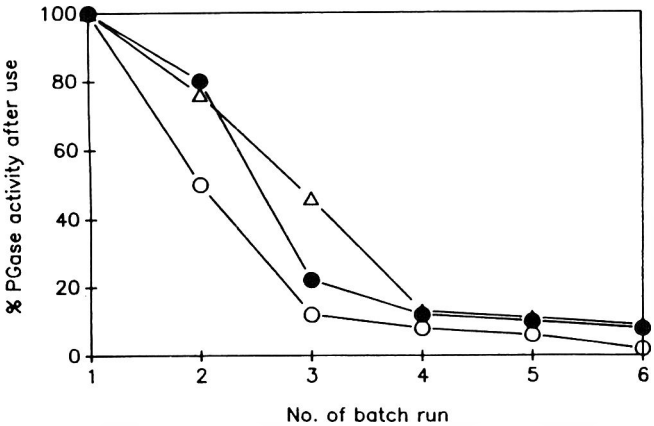

Fig. 1—Activity of PGase immobilized on calcium phosphate gel toward soy protein hydrolysate upon multi-use at 30°C for 30 min: (○) Undialyzed peptone in 0.050–0.085 M phosphate buffer, pH 6.0–7.5; (●) Peptone in 0.025M phosphate buffer, pH 6.5 or (△) peptone in 0.025M phosphate buffer, pH 6.1, both dialyzed against water in a 1,000 cutoff membrane.

30°C for 16 hr or longer without any permeation or deterioration in activity. With the S1-Y30 membrane complete recoveries of PGase could be obtained with each sequential run, resulting in complete conversion of glutamine amides to carboxyl groups and allowing multiple reuse of PGase. Use of this process would increase productivity of the system and result in an enzyme-free product.

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Preservative and Temperature Effect on Growth of Three Varieties of the Heat-Resistant Mold, *Neosartorya fischeri*, as Measured by an Impedimetric Method

P.V. NIELSEN

ABSTRACT

The combined effects of inoculum amount, temperature, pH, and food preservatives on growth of three varieties of heat-resistant mold *Neosartorya fischeri* cultured on a fruit juice-like medium was investigated. *N. fischeri* var. *glabra* growth occurred at 100 mg sodium benzoate/L at pH 3.5, a level that completely inhibited outgrowth of the other varieties. All were similarly affected by sulfur dioxide; completely inhibited by 300 mg/L at pH 3.5. The inhibitory effect of preservatives was strongly affected by pH, inoculum amount, and temperature. Growth was significantly delayed when temperature was decreased from 32 to 25 to 18°C or pH from 4.5 to 3.5. This is the first report on the use of impedance microbiology to study the effects of environmental factors on molds.

INTRODUCTION

NEOSARTORYA FISCHERI (anamorph *Aspergillus fischerianus*) is one of the most frequently reported heat-resistant molds causing spoilage in fruit juices and other heat processed fruit-based products (Beuchat, 1986). As the heat-resistant ascospores are widely distributed in soil, the spoilage problems predominately occur in products containing fruits harvested from or near the ground (Hocking and Pitt, 1984).

The species *N. fischeri* consists of three distinct varieties, *N. fischeri* var. *fischeri, N. fischeri* var. *glabra* and *N. fischeri* var. *spinosa* having clearly different ascospore ornamentations and profiles of mycotoxins and other secondary metabolites (Kozakiewicz, 1989; Samson et al., 1990; Nielsen and Samson, 1991). The extremely heat-resistant ascospores are biconvex with two equatorial crests. In varieties *glabra* and *spinosa* ascospore surfaces are smooth and spinulose respectively, whereas variety *fischeri* has ascospores with a characteristic reticulate surface. *N. fischeri* var. *fischeri* has several similarities with *Aspergillus fumigatus*. Both produce the strongly tremorgenic fumitremorgins, which have been proven to cause ryegrass staggers (Patterson et al., 1981) and acute toxic syndrome in beef cattle being fed molded corn silage (Cole et al., 1977). They also produce conidia abundantly and are known to cause mycosis in man (Raper and Fennell, 1965; Coriglione et al., 1990). The other varieties have much more restricted conidiogenesis. Varieties *fischeri* and *spinosa* produced high amounts of the potentially tremorgenic tryptoquivalines, while none of the large number of compounds produced by variety *glabra* have been identified as known mycotoxins (Samson et al., 1990).

Only the varieties *glabra* and *spinosa* have positively been isolated from spoiled heat-processed fruit-based products (Kavanagh, 1963; McEvoy and Stuart, 1970; Jesenska et al., 1984), while several other authors did not report the *N. fischeri* variety causing food spoilage (Baggerman, 1984; Cartwright and Hocking, 1984; Hocking and Pitt, 1984; Jesenska and Petrikova, 1985; Scott and Bernard, 1989; Splittstoesser and Splittstoesser, 1977; Splittsoesser and Churey, 1989). A number of these isolates were reexamined by Samson et al. (1990) and Nielsen and Samson (1991) and none belonged to variety *fischeri*. Recent findings in our laboratory have shown that the *fischeri* variety tends to dominate in soil (unpublished results). Kavanagh et al. (1963) also found var. *fischeri* to be numerous in soil but could not recover if from heat processed fruit products.

Studies of the food spoiling capacity of these organisms have so far only been carried out at species level (i.e. neglecting to differentiate between varieties). The organism used to study growth and mycotoxin production by *N. fischeri* under a wide range of environmental conditions by Nielsen et al. (1988, 1989a, b) has later been identified as variety *fischeri*, which is now considered to be less food relevant than the others (Samson et al., 1990). It is therefore of great interest to investigate whether the three varieties of *N. fischeri* have different resistance towards commonly used food preservation techniques or other factors in the composition of a food product. This information may be valuable when handling fruit products and to explain differences in food spoiling capacity.

This study was designed to evaluate how growth of the varieties of *N. fischeri* was affected by the combined effects of inoculum amount, temperature, pH, sodium ascorbate and each of the food preservatives sodium benzoate, potassium sorbate and sulfur dioxide.

MATERIALS & METHODS

Preparation of inoculum

Organisms are listed in Table 1. The test molds were cultivated for 14 days on oat meal agar (OA) plates (Samson and van Reenen-Hoekstra, 1988). Ascospores were harvested by flooding the surface of the mycelial mat with 2.5 mL of dilution water (1g peptone, 8.5g sodium chloride and 1g Tween 80/L) and gently rubbing the mold surface with a sterile bent glass rod. Suspensions of asci were pipetted into a sterile glass tube. This procedure was repeated, combining the two suspensions, which were treated for 2 min in an ice cooled water bath sonicator to disrupt the asci and free ascospores. Cleistothecia and intact asci were allowed to settle for 2 to 3 min. before ascospores in suspension were filtered through sterile glass wool into a sterile tube. The ascospore suspensions were diluted to 10^6 ascospores per mL as determined by counting chamber and heat treated for 15 min at 75°C. This was done to inactivate conidial spores and mycelial fractions and to activate the ascospores. Three additional inocula were obtained by serial dilutions of the heat treated ascospore suspension to 10^5, 10^4 and 10^3 ascospores per mL. The number of viable asco-

Table 1—Organisms examined[a]

Neosartorya fischeri			
var. *fischeri*	IBT	3023	ex soil Denmark
	IMI	16143	ex soil UK
var. *glabra*	IMI	102173	ex canned strawberries, Eire
	IBT	3004	ex soil Denmark
var. *spinosa*	IBT	3001	ex soil Denmark
	CBS	483.65	ex soil Nicaragua
	CBS	297.67	ex soil Pakistan

[a] Culture collections: IBT: the collection at the Department of Biotechnology, Technical University Denmark; CBS: Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; IMI: International Mycological Institute, Kew, UK.

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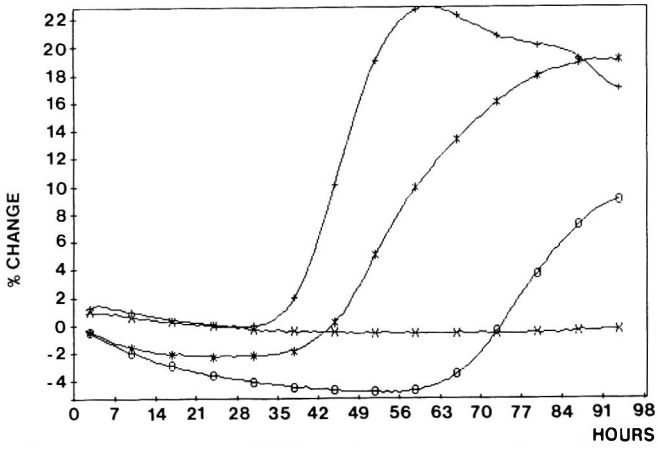

Fig 1—Capacitance change in a fruit juice-like media, PYS at pH 4.5 and 32°C, with time after inoculation of 10^3 heat inactivated ascospores, N. fischeri *var.* glabra *(IMI 102173). Curves represent increasing concentrations of potassium sorbate in mg/L 0 (+) 25 (*), 50 (O) and 100 (X).*

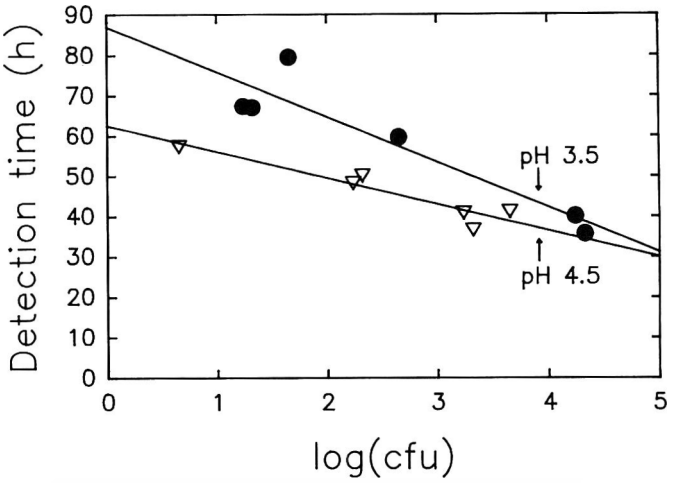

Fig 2—Detection time (DT) as related to the logarithm of the initial numbers of viable ascospores of N. fischeri *var.* glabra *(IMI 102173), at pH 3.5 (●) and 4.5 (▽).*

spores was determined by enumeration on PYS (see below) at pH 4.5 and pH 3.5.

Microbial monitoring system

Experiments were carried out in a Bactometer® B123-2 (bioMérieux UK ltd., UK). The system consisted of 2 Bactomatic Processing Units (BPU) each having 2 accurately temperature-controlled incubators. Each incubator held 4 disposable Bactometer modules of 16 wells. The Bactometer was capable of monitoring fungal growth in all 256 wells simultaneously, by applying a sinusoidal AC current across two stainless steel electrodes mounted in the bottom of each well and measuring the capacitance. At 6 min intervals, the instrument made 1 reading in each well and stored the data. An advanced algorithm determined the detection time based on rate and magnitude of change in capacitance.

Substrate

Potassium dihydrogen phosphate yeast extract sugar agar (PYS) was used as a basic medium throughout. This medium was designed to contain a mixture of sugars typical of most fruit juices. It consists of (per L of distilled water) potassium dihydrogen phosphate, 10g; yeast extract (Difco), 7.5g; glucose, 30g; fructose, 30g; sucrose, 40g; and agar 20g. The medium was autoclaved at 121°C for 15 min. Filter sterilized (0.22 mμ) stock solutions of sodium ascorbate, sodium benzoate, potassium sorbate and sulfur dioxide (as sodium metabisulfite) were prepared and added to PYS to give 0 and 200 mg/L of sodium ascorbate (the level allowed in fruit juices in Denmark) in combination with 0, 25, 50 and 100 mg/L of sodium benzoate or potassium sorbate or 0, 50, 100 and 200 mg/L of sulfur dioxide. pH was adjusted to 3.5 and 4.5 with sterile HCl and media were poured into disposable Bactometer modules, 0.80 mL in each well. In a preliminary experiment 100 and 300 mg/L of each preservative were added to PYS and pH adjusted to 3.5.

Inoculation procedure

Aliquots (100 μL) of the ascospore suspensions (10^6, 10^5, 10^4, and 10^3 cfu/mL) were dispensed into prefilled wells and the modules were tipped to distribute the ascospore suspension on the agar surface. The number of ascospores in the Bactometer measuring cells were consequently 10^5, 10^4, 10^3, and 10^2 CFU/well. Modules were incubated at 18, 25 and 32°C for 100 hr (4 days) in the Bactometer whereupon they were placed in separate incubators for additional 14 days.

Analyses

Detection time (= time until growth was evident) was during the first 4 days recorded by the Bactometer (one reading every 6 min) and for the last 14 days by visual inspection every day. Data obtained in the last period was consequently more variable.

Statistical analyses

The experiment was designed as a reduced factorial experiment at two levels ($1/4 \times 2^7$), were inoculum amount was confounded with 4 factor interactions (Box et al., 1978). The 4 levels of preservative concentration and inoculum size were obtained by combining 2 factors on 2 levels. This was done to simplify calculations. The temperature effect was examined by comparing 2 temperatures at a time (25 with 18°C and 25 with 32°C). Due to the number of tests the experiments were divided into 3 parts (1 with each preservative). Detection time on media without preservatives in the 3 parts were used to calculate growth rate and length of lag phase. Analyses of the effect of preservatives were performed using the principles of Yates algorithm and reverse Yates algorithm described by Box et al. (1978) and the MGLH module in the software package SYSTAT (Systat Inc., Il, USA). Factors at 4 levels were analyzed as orthogonal polynomials (Hicks, 1982).

RESULTS & DISCUSSION

CULTIVATION of *Neosartorya fischeri* varieties on the juice-like medium PYS resulted in consistent and rapid capacitance change and good production of secondary metabolites. Figure 1 shows the change in electrical capacitance during growth of *N. fischeri* var. *glabra* on PYS supplemented with various concentrations of potassium sorbate at pH 4.5. Detection times were computed automatically by the Bactometer by analyzing the rate and magnitude of change in capacitance. When the criteria set in the detection time algorithm were met, growth was detected. Detection time may be determined visually from the figure as the point were the curves start to accelerate. Detection time increased with increasing preservative concentration, i.e. the mold grew slower.

Within a certain range, a linear relationship exists between the logarithm of colony forming units, log(CFU) and the detection time (DT) as measured by the Bactometer (Fig. 2). Watson-Craik et al. (1990) also found a linear relationship between detection time and log(CFU) for a high number of food spoilage fungi. However the relationship was species-specific due to differences in growth rate. Firstenberg-Eden and Eden (1984) found that change in capacitance followed the metabolism of media components and hence depended on the biomass and metabolic rate/mass unit. After germination and lag phase, the mold biomass will increase exponentially at a fixed rate until a deficiency of 1 or more media component occurs or until self inhibition takes place. This will most likely not occur before the detection of growth, as mold growth first became visible shortly after detection. The capacitance change caused by fungal growth would therefore increase exponentially resulting in a sharp increase in capacitance when suffi-

Table 2—Growth rate (μ) of Neosartorya fischeri varieties (hr^{-1}) at 3 temperatures and 2 pH levels (3.5 and 4.5).

		18°C		25°C		32°C	
		3.5	4.5	3.5	4.5	3.5	4.5
N. fischeri var.							
fischeri	IBT 3032	0.35	0.16	0.27	0.40	0.65	0.68
glabra	IMI 102173	0.32	0.21	0.20	0.31	0.61	0.53
glabra	IBT 3004	0.24	0.22	0.21	0.35	0.46	0.49
spinosa	IBT 3001	0.33	0.15	0.31	0.39	0.46	0.93

Table 3—Length of lag phase (hr) for N. fischeri varieties at 3 temperatures and 2 pH levels (3.5 and 4.5)[a]

		18°C		25°C		32°C	
		3.5	4.5	3.5	4.5	3.5	4.5
N. fischeri var.							
fischeri	IBT 3023	128.3	63.4	33.6	21.9	23.1	12.6
glabra	IMI 102173	92.0	50.7	9.5	16.0	19.7	9.9
glabra	IBT 3004	68.8	46.6	8.7	16.8	13.4	6.6
spinosa	IBT 3001	92.1	48.3	21.4	18.9	14.2	15.2

[a] Estimated as detection time for cfu = 10^7.

cient biomass was reached, and the instrument "signals" detection. This will thus occur earlier when the inoculum contains a high CFU as compared to a lower, as the required biomass would be reached earlier. The slopes on the regression lines in Fig. 2 represent the increase in detection time at the two pH values, when the initial number of ascospores was increased by factors of 10 (1 log unit). We assumed the fungal biomass was increasing exponentially from the end of the lag phase until inhibition occurred, sometime after detection, and the metabolic activity per biomass was constant during that time. Then the rate of increase in metabolic activity, μ, may be expressed as $\mu = -\ln(10)/\alpha$ where α is the slope. Growth rate of the organism in terms of rate of increase in biomass/unit time is also μ, as the metabolic activity is proportional to biomass. Doubling time, g, for the metabolic activity is then: $g = \ln(2)\mu = -\alpha \log(2) \approx -\alpha \cdot 0.301$. This number represents the delay in detection time when CFU is diminished by one half. Figure 2 shows a steeper slope at pH 3.5 than at 4.5 indicating a slower growth rate at the lower pH.

Table 2. lists growth rate, μ, for each of the 3 varieties at the 3 temperatures and the 2 pH levels. Within each treatment no significant difference ($P < 0.05$) between varieties was observed. At 25°C the organisms appeared to grow slower at pH 3.5 than at pH 4.5, whereas growth rates at 18°C appeared higher at pH 3.5 than at 4.5 and only variety *spinosa* appeared different in growth at 32°C. However, none of those apparant differences was significant ($P < 0.05$). Temperature had a much stronger effect especially at pH 4.5, where growth rates more than doubled with increased temperature from 18 to 32°C.

Temperature also had a clear effect on detection time when a given medium was inoculated with the same number of ascospores as shown in Fig. 3. Data for the three varieties have been combined, as dissimilarities between varieties were insignificant. The difference in detection time at the two pH levels may account for differences in lag time as growth rates only were moderately affected by pH. The same may be the case with the large differences in detection time at different temperatures.

Preliminary experiments have shown that the straightline relationship between log(CFU) and detection time only existed within a limited range (about 10^1 to 10^6 ascospores/well). Above that inoculum level the curves leveled and the detection time converged to a common point. This detection time would be a measure of the length of the lag phase as the instrument detects immediately after the ascospores have germinated and growth has begun. An estimate of that value may be obtained by extrapolating the detection time for a well containing 10^7 ascospores. Table 3 lists the estimated length of lag phase for each variety. The general tendency was that the lag phase decreased when pH was increased from 3.5 to 4.5 and when the temperature was increased from 18 to 25 to 32°C. Out-

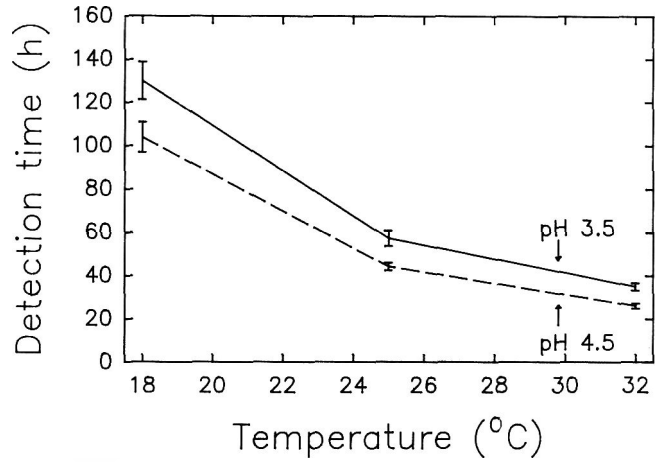

Fig 3—Effect of temperature on detection time, (DT) at a fixed initial number of viable ascospores (log(CFU) = 3.0) at pH 3.5 (———) and 4.5 (– – – –). Bars represent 95% confidence limits.

growth of variety *fischeri* ascospores were clearly delayed as compared to other varieties at 18°C. This may favor growth of the varieties *glabra* and *spinosa* over variety *fischeri* if present in a mixed culture.

More rapid growth at higher temperatures is one of the characteristics of *Aspergillus fumigatus* group under which *Neosartoya fisheri* was included by Raper and Fennel (1965). Most fungi in this group grow well at 37°C, some approaching a true thermophilic range. Samson and van Reenen-Hoekstra (1988) reported the optimum temperature for growth was 26 to 45°C. This range was narrowed somewhat by Nielsen et al. (1988) who reported the temperature optimum for growth was near 37°C.

Sodium ascorbate (200 mg/L) did not affect the detection time. However, this did not exclude the possibility that ascorbate could have an effect if aeration had been restricted, as ascorbate usually is added to products to inhibit oxidation.

Preservative concentration and pH had a clearly significant ($P < 0.05$) effect on detection time. However, the varieties of *N. fischeri* were not equally affected as shown in Table 4.

Sodium benzoate. Outgrowth of ascospores of variety *fischeri* and variety *spinosa* was not observed at sodium benzoate concentrations higher than 50 mg/L pH 3.5 within the 20 day incubation. Growth of variety *glabra* was evident, however, at 100 mg/L of sodium benzoate, pH 3.5 after 4 to 11 days depending on temperature and inoculum size. No growth was observed at 300 mg/L, 25°C. Difference between varieties may be seen in greater detail in Fig 4, showing the estimated de-

Table 4—Minimal preservative concentration (mg/L) suppressing growth for more than 18 days at 3 temperatures at 2 pH levels (3.5 and 4.5)

	Sodium benzoate						Potassium sorbate						Sulfur dioxide					
	18°C		25°C		32°C		18°C		25°C		32°C		18°C		25°C		32°C	
	3.5	4.5	3.5	4.5	3.5	4.5	3.5	4.5	3.5	4.5	3.5	4.5	3.5	4.5	3.5	4.5	3.5	4.5
Neosartorya fischeri																		
var. *fischeri*																		
IBT 3023	100	>100	100	>100	50	>100	25	100	300	50	100	>100	200	>200	300	>200	200	>200
IMI 16143	50	>100	50	>100	100	>100	25	100	50	>100	100	>100	200	>200	200	>200	100	>200
var. *glabra*																		
IMI 102173	>100	>100	300	>100	>100	>100	50	>100	300	>100	50	>100	200	>200	300	>200	200	>200
IBT 3004	>100	>100	300	>100	>100	>100	50	>100	300	>100	50	>100	>200	>200	300	>200	200	>200
var. *spinosa*																		
IBT 3001	100	>100	100	>100	100	>100	50	>100	300	100	50	>100	>200	>200	300	>200	100	>200
CBS 483.65	100	>100	100	>100	100	>100	50	100	50	>100	100	100	200	>200	200	>200	200	>200
CBS 297.67	100	>100	100	>100	100	>100	25	100	50	100	100	100	200	>200	200	>200	200	>200

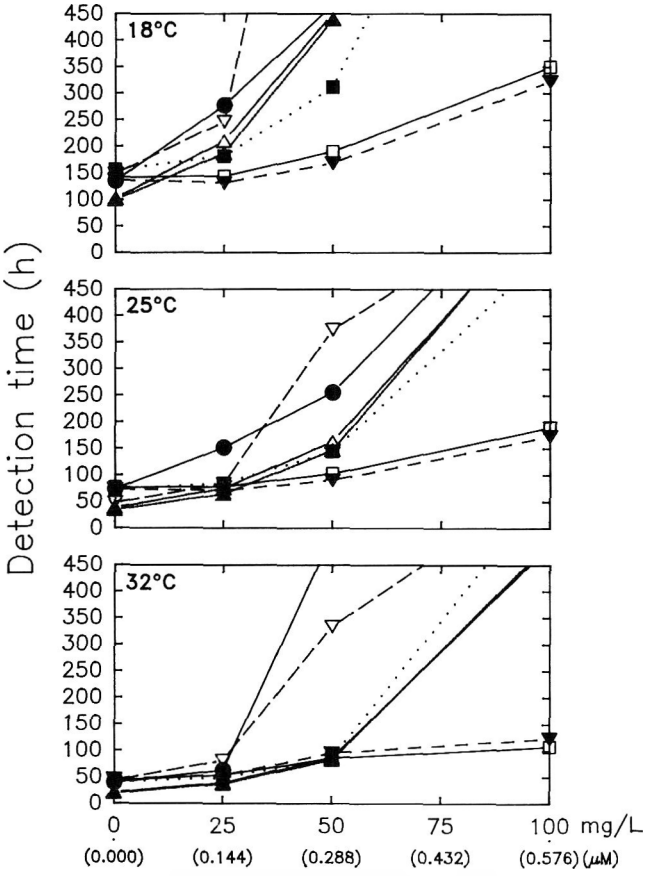

Fig 4—Sodium benzoate effect on detection time at pH 3.5 at 18, 25, and 32°C. DT estimated for log(CFU/mL) = 3.0. Isolates were N. fischeri *var.* fischeri *IBT 3023* (●———●); *var.* fischeri *IMI 16143* (▽– –▽); *var.* glabra *IMI 102173* (▼– –▼); *var.* glabra *IBT 3004* (□———□); *var.* spinosa *IBT 3001* (■· · ·■2); *var.* spinosa *CBS 483.65* (△———△); *var.* spinosa *CBS 297.67* (▲–·–·–▲). *Molar concentrations of undissociated acid (μM) were calculated on the basis of* pK_a = 4.19.

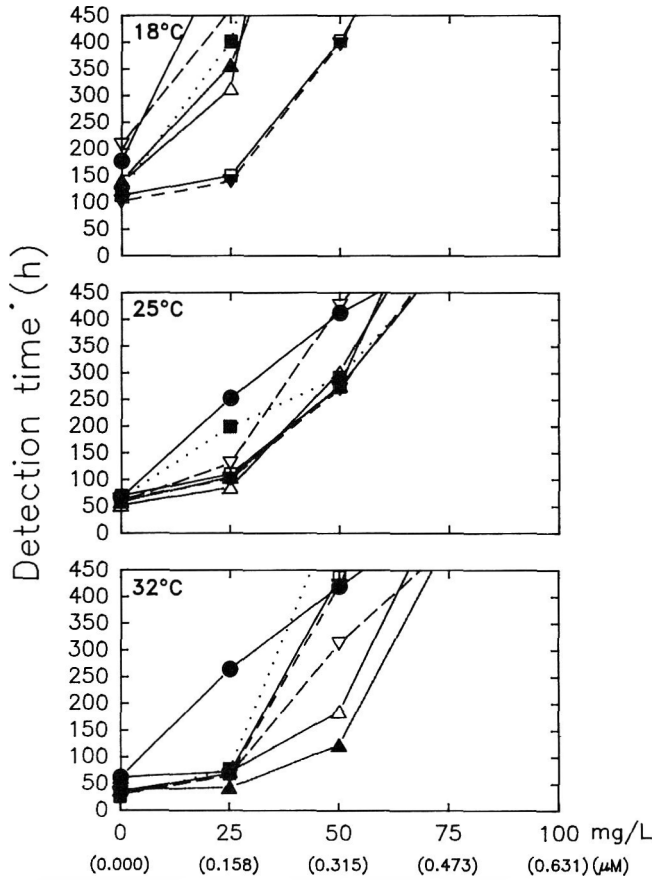

Fig 5—Potassium sorbate effect on detection time at pH 3.65 at 18, 25, and 32°C. Molar concentrations of undissociated acid (μM) were calculated on the basis of pK_a = 4.76. See legends for Fig. 4.

tection times for all isolates at pH 3.5. Each curve was estimated from 32 readings. As the variation in detection time increased with time, some uncertainties were expected at longer detection times. The isolates of variety *fischeri* were somewhat more inhibited by sodium benzoate than variety *spinosa*, especially at 32°C. Both were clearly less resistant than *glabra* which was almost unaffected by preservative concentration. The estimated detection times at pH 4.5 are not shown as only a slight delay in detection time was observed. The minimum preservative concentration (mg/L) inhibiting growth is listed in Table 4. Based on the dissociation constants of benzoic acid, pK_a = 4.19, the molar concentrations of undissociated acid at an initial concentration of 100 mg sodium benzoate/L are 0.58 and 0.23 μmol/L at pH 3.5 and 4.5, respectively.

Potassium sorbate was generally more inhibitory than sodium benzoate but some inconsistency was observed between isolates of variety *fischeri* and *spinosa*. Inhibition by sorbate at 18°C, pH 3.5 was parallel to but somewhat stronger than by benzoate. Figures 5 and 6 show the estimated detection times at pH 3.5 and 4.5. Molar undissociated acid calculated on the basis of the dissociation constant of sorbic acid, pK_a = 4.76, is presented in the figures to ease comparison. Variety *glabra* showed a clearly higher resistance at 18°C than the other varieties, which in turn were almost equally affected. At increasing temperatures the inhibition curve for isolates of variety *glabra* only shifted moderately while the other varieties became much less inhibited. The two isolates of variety *fis-*

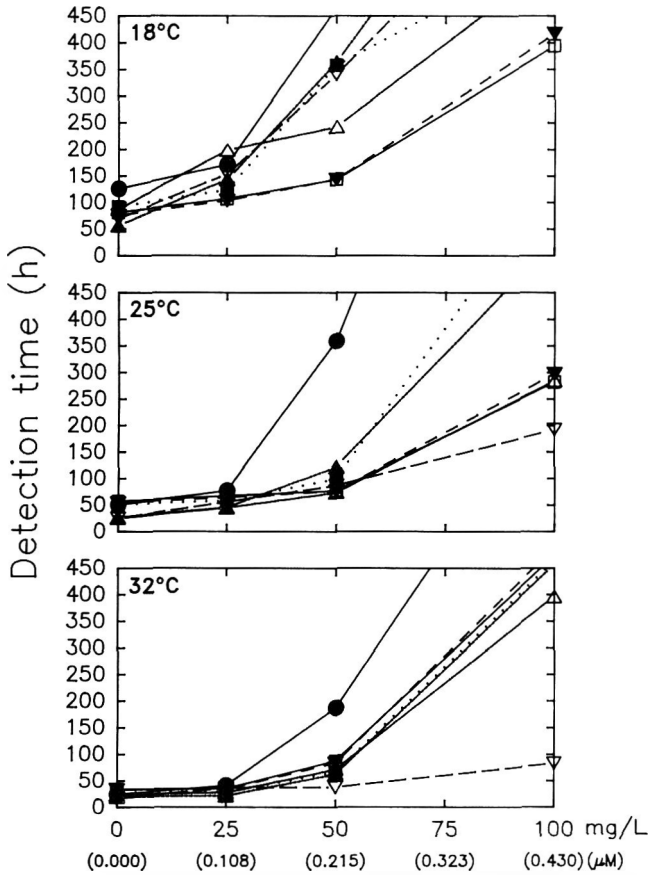

Fig 6—Potassium sorbate effect on detection time at pH 4.5 at 18, 25, and 32°C. Molar concentrations of undissociated acid (μM) were calculated on the basis of pK_a = 4.76. See legends for Fig. 4.

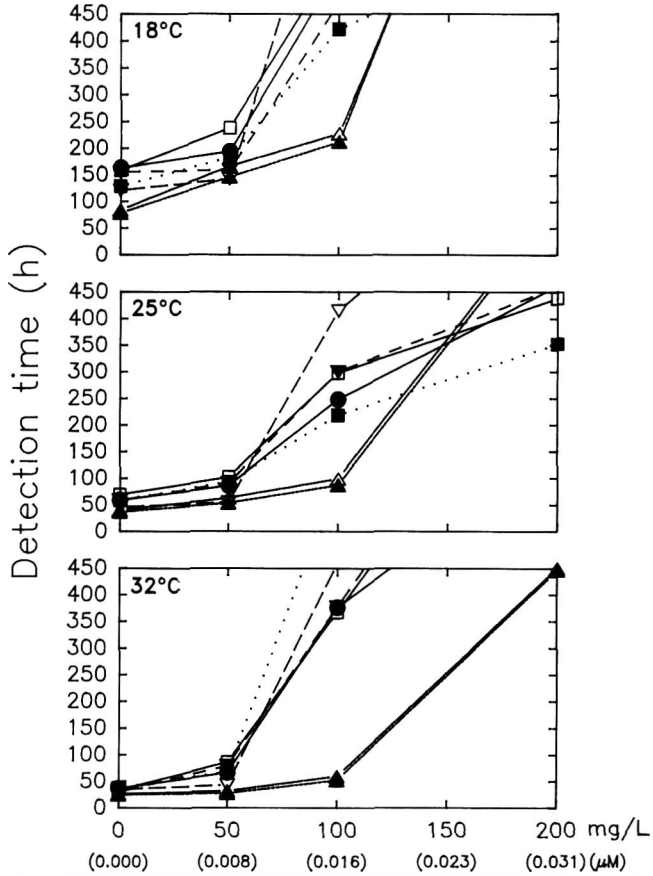

Fig 7—Sulfur dioxide effect on detection time at pH 3.5 at 18, 25, and 32°C. Molar concentrations of undissociated acid (μM) were calculated on the basis of pK_a = 1.81. See legends for Fig. 4.

cheri showed a marked difference in resistance at pH 4.5 especially at 25 and 32°C, where IMI 16143 showed much higher resistance than IBT 3023. The strain of variety *spinosa*, IBT 3001, also showed somewhat higher resistance than the other strains of variety *spinosa*.

Sulfur dioxide. All varieties were almost equally affected by sulfur dioxide. 200 mg/L sulfur dioxide were sufficient to delay ascospore germination and subsequent outgrowth for more than 20 days at pH 3.5, 32°C (Fig. 7). At 25°C all isolates were detected after 10 days on the same media when inoculated with 10^5 CFU, but not within 20 days when inoculated with less than 10^3 CFU. None was able to grow at 300 mg/L. At 18°C only 1 isolate each of variety *glabra* and variety *spinosa* was detected within 20 days at that concentration (Table 4). Two isolates of variety *spinosa* (CBS 297.65 and CBS 483.65) showed a somewhat higher resistance towards sulfur dioxide at pH 3.5 than the other isolates especially at 32 and 25°C, (Fig 7). The estimated detection times at pH 4.5 are not shown, as detection time in general less than doubled when sulfur dioxide was increased from 0 to 200 mg/L. Exceptions were the growth of variety *glabra* isolates at pH 4.5 and 32°C, which were suppressed for more than 20 days when inoculated with low numbers. However this organism was only slightly inhibited when the inoculum was more concentrated. The molar concentrations of undissociated sulfurous acid at pH 3.5 and 4.5 calculated on the basis of the dissociation constant, pK_a = 1.81, were 0.062 and 0.0064 μmol/L respectively at an initial concentration of 200 mg/L.

In general variety *fischeri* growth was more restricted than growth of other varieties. Variety *glabra* grew at highest concentrations of sorbate and benzoate whereas variety *spinosa* was somewhat more resistant towards sulfur dioxide. The much lower resistance at pH 4.5 as compared to 3.5 was in good agreement with the generally accepted theory that the undissociated molecule was the major active antifungal agent (Cruess et al., 1931; Booth and Kroll, 1989). The lower dissociation constants of sulfurous and benzoic acid (pK_a: 1.81 and 4.19 respectively) as compared to sorbic acid (pK_a: 4.76) may also explain why sorbate still was quite effective at pH 4.5. An important general observation was that resistance towards preservatives tended to increase with inoculum amount. In other words a highly contaminated product is more difficult to preserve than a "clean" product. However it is not clear whether outgrowth at low CFU was blocked completely or merely restrained, i.e. outgrowth may also occur at low CFU after prolonged incubation.

The fungistatic effect of sodium benzoate towards *N. fischeri* var. *fischeri* on Czapek yeast extract agar (CYA) at pH 3.5 (growth at 50 but not at 75 mg/L, Nielsen et al., 1989a) was similar to data found for varieties *fischeri* and *spinosa* in our study. King and Halbrook (1987) reported that *Talaromyces flavus* cultured on potato dextrose agar at pH 3.5 was totally inhibited at 100 mg sodium benzoate/L. Other heat-resistant molds (*Byssochlamys fulva* and *B. nivea* are like *N. fischeri* var. *glabra* able to withstand somewhat higher concentrations of benzoate. King et al. (1969) reported that *B. fulva* grew in reconstituted grape juice at room temperature containing 1000 mg sodium benzoate/L. Beuchat (1976) found that *B. nivea* tolerated up to 1000 mg/L when cultured in grape juice at 30°C, while Roland et al. (1984) found the same isolate was completely inhibited for more than 25 d in grape juice containing 500 mg sodium benzoate/L. In apple juice growth was observed at 500 mg/L (Roland and Beuchat, 1984).

The isolates we examined were generally more inhibited by

sorbate than benzoate at pH 3.5. The difference was most pronounced at 18°C whereas only *N. fischeri* var. *glabra* showed a clear difference at 25°C. Potassium sorbate was also more inhibitory than sodium benozate to the heat resistant molds in genus *Byssochlamys*. King et al. (1969) found that *B. fulva* growth could be controlled by 500 mg potassium sorbate/L. Beuchat (1976) found complete inhibition at 400 mg/L in grape juice and at 200 mg/L in PDA, pH 3.5. 150 mg potassium sorbate/L delayed growth of *B. nivea* for more than 25 d in apple and grape juice when incubated at 21°C, but not at 30 and 37°C (Roland and Beuchat, 1984; Roland et al., 1984).

All varieties of *N. fischeri* we examined grew at sulfur dioxide concentrations as high as 200 mg/L, pH 3.5, but not 300 mg/L. When cultured on CYA, pH 3.5 growth of *N. fischeri* var. *fischeri* was not observed at concentrations higher than 100 mg/L (Nielsen et al., 1989a). *B. nivea* did not grow at sulfur dioxide concentrations higher than 75 mg/L in apple juice with pH around 3.5 (Roland and Beuchat, 1984). In grape juice growth was delayed but occurred at 100 mg/L (Roland et al., 1984). Growth of the same *B. nivea* isolate was observed in grape juice containing 200, but not 300 mg sulfur dioxide/L when incubated for 60 d (Beuchat, 1976). However, growth was completely inhibited when the organism was cultured on PDA, pH 3.5 containing 50 mg sulfur dioxide/L. The large differences in sulfur dioxide tolerance observed for the same organism cultured on different substrates was most likely caused by the binding of sulfur dioxide to anthocyanins and other flavanoids, sugars and other aldehydes (Booth and Kroll, 1989). Data from our study may be considered representative for fruit juices, as the sugar and protein content of the basic medium used were similar to contents of fruit juices.

CONCLUSION

N. FISCHERI VAR. *GLABRA* and var. *spinosa* were demonstrated to germinate and grow at higher preservative concentrations than *N. fischeri* var. *fischeri*. The preservative levels required to control outgrowth were comparable to those required for other heat resistant fungi. Also with respect to growth rate and lag phase at 18°C the *fischeri* variety was slower than the others. This may in part explain why that variety has not been identified as a spoiler of heat-preserved food products. Other factors of importance may be atmospheric composition and heat-resistance, which currently are under study in our laboratory.

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Heat Resistance of *Salmonella typhimurium* and *Listeria monocytogenes* in Sucrose Solutions of Various Water Activities

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ABSTRACT

The heat resistance of *Salmonella typhimurium* was determined in sucrose solutions with a_w ranging from 0.98–0.83. The $D_{65.6°C}$ ranged from 0.29–40.2 min (>100-fold increase), with z value 6.5–7.7°C. *S. typhimurium* was also heated in four chocolate syrups purchased at retail. The $D_{65.6°C}$ ranged from 1.2–3.2 min while the a_w of the syrups ranged from 0.75–0.84. The heat resistance of *Listeria monocytogenes* was determined in sucrose solutions with a_w ranging from 0.98–0.90. The $D_{65.6°C}$ value shifted from 0.36–3.8 min (10-fold increase); the z value ranged from 7.6–12.9°C.

INTRODUCTION

SALMONELLA and *Listeria monocytogenes* are bacteria of serious concern to the food industry. *L. monocytogenes* can grow at refrigeration temperatures (Rosenow and Marth, 1987), survive drying (Doyle et al., 1985) and freezing (Golden et al., 1988) and tolerate highly alkaline conditions (Gray and Killinger, 1966). *L. monocytogenes* can also cause severe morbidity and mortality in particular subpopulations, most notable being the immunocompromised, neonates, and fetuses of pregnant women. The mortality rate in recent foodborne outbreaks has ranged from 29–44%. It has been estimated that 1,700 persons in the U.S. suffer *Listeria* infections annually (Gellin and Broome, 1989), of which 400 may die. Due to such statistics, the FDA and USDA have taken a strong regulatory position on the occurrence of this organism in the food supply, resulting in several product recalls.

Salmonella continues to be a leading cause of foodborne disease in the U.S. Survival of *Salmonella* spp. in chocolate products is a major concern of the confectionery industry. The increased thermal resistance of *Salmonella typhimurium* (Baird-Parker et al., 1970; Goepfert et al., 1970; Gibson, 1973; Corry, 1974) and *L. monocytogenes* associated with decreasing water activity (a_w) emphasizes the need to define adequate thermal processes to eliminate both *Salmonella* and *L. monocytogenes*.

The industry, therefore, faces a challenge in controlling the presence of these organisms in food production facilities and in eradicating them from finished products. Sporeforming organisms such as *Clostridium botulinum* do not grow at a water activity <0.93 after mild heat treatment (Denny et al., 1969). Therefore thermal processes for intermediate moisture foods are usually based on inactivation of other organisms of public health concern, such as *Salmonella* or *L. monocytogenes*. Both *Salmonella* and *L. monocytogenes* can remain viable at water activity values <0.93. Thus the potential for infection when such products are consumed becomes a factor to be considered when establishing processing or handling practices. Because water activity (a_w) is used in combination with other factors to control pathogenic and spoilage microorganisms, other processes such as heating may be necessary to inactivate potentially pathogenic but non-multiplying organisms. Therefore, our objective was to determine the effect of a_w on heat resistance of *S. typhimurium* and *L. monocytogenes*.

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MATERIALS & METHODS

Organisms and culture conditions

S. typhimurium ATCC 13311 was obtained from the American Type Culture Collection, Rockville, MD. *S. typhimurium* was grown to stationary phase in 10–20 mL brain heart infusion broth (35°C for 24 h). The cells were washed in 0.1M phosphate buffer (pH 6.5) and suspended in the test sucrose solution or 1 of 4 commercial chocolate syrups and allowed to adjust to the osmotic environment for 3 hr prior to heating. Counts on suspensions immediately after inoculation and at the end of 3 hr indicated initial loss of viability of 1–2 log cycles due apparently to osmotic effects (data not shown). Therefore the procedure was adjusted to allow for osmotic equilibration of cells after suspension in the test solution. Initial counts for calculating D values were determined via plating on trypticase soy agar after a 3 hr period to allow osmotic equilibration.

L. monocytogenes (Scott A, a clinical isolate from a 1983 Massachusetts outbreak (Fleming et. al, 1985)) was cultured at 30°C in 10–20 mL trypticase soy broth plus 0.6% yeast extract for 18 hr. The stationary phase cells were centrifuged and washed in 0.1M phosphate buffer (pH 7.0). The cells were suspended in the test sucrose solution and allowed to equilibrate for 3 hr. Initial counts were then determined using trypticase soy agar containing 0.6% yeast extract.

Water activity of heating menstrua

Preliminary formulations of sugar solutions were based on results reported by Gibson (1973); the water activity was determined and adjustments were made as necessary to achieve appropriate a_w levels. On the day of the test sucrose (heated 1 hr at 115.6°C to destroy competing microflora) was added to phosphate buffer to adjust water activity to 0.98 to 0.83. The water activity of the sugar solutions and the chocolate syrups was measured in duplicate with a Rotronics Hygroskop DT electric hygrometer (Kaymont Instrument Corp., Huntington, NY) or a Beckman Hygroline electric hygrometer (Beckman Instruments, Cedar Grove, NJ). The equipment was calibrated with saturated salt solutions from which standard curves were generated based on published a_w values (Greenspan, 1977). The chocolate syrups had been irradiated to destroy competing organisms before the thermal death time (TDT) studies were conducted.

Thermal death time (TDT)

TDT testing was carried out by a modification of procedures outlined by the National Canners Association (1968). Cells of *S. typhimurium* or *L. monocytogenes* were diluted in 200–500 mL of sugar solution or chocolate syrup to a concentration of 1×10^7 CFU/mL for *S. typhimurium* and 1×10^5 CFU/mL for *L. monocytogenes* as determined by counts at the end of equilibration. After equilibration to the osmotic environment, suspensions were distributed in 1 mL aliquots to sterile Pyrex glass TDT tubes (8 mm i.d. × 10 cm) in preparation for heating. The tubes were sealed using an oxygen torch. Five to 10 tubes/time interval were tested. TDT tubes in metal holders strung on metal rods were immersed in a heated oil bath. All tubes for a single temperature were immersed at the same time. At the end of each time

Table 1—D and z values of S. typhimurium *strain ATCC 13311 in sucrose solutions of various water activities*

	D-value (minutes)					
	Temperature (°C)					z value
a_w	65.6	68.3	71.1	73.9	76.7	(°C)
0.98	0.29	0.12	0.05	0.02	0.01	7.6
0.94	1.4	0.61	0.27	0.12	0.05	7.7
0.89	4.8	1.9	0.74	0.29	0.12	6.9
0.85	19.2	7.3	2.8	1.1	0.40	6.5
0.83	40.2	17.4	7.5	3.3	1.4	7.6

interval a rod containing the 5–10 replicates was removed and the tubes immediately immersed in an ice water bath. Temperature data were collected with a copper constantan thermocouple located in the approximate center of the inoculum in a TDT tube strung on the rod containing the tubes for the longest time interval (last to be removed from the bath). Data from the thermocouple were recorded at 10 sec intervals using a Kaye Digistrip II potentiometer and a DEC Mini-MINC computer.

After heating and cooling, each tube was opened and the suspension poured into 9 mL of recovery medium. Suspensions containing *S. typhimurium* were cultured in lactose broth and incubated at 30°C. The heat stressed *L. monocytogenes* were resuscitated in tryptose phosphate broth with 1% pyruvate and incubated at 30°C. After 48 hr any lactose broth tubes showing signs of growth were streaked onto Hektoen Enteric Agar for confirmation of the presence of *Salmonella*. After 48 hr, 1 wk and 2 wk incubation, positive tryptose phosphate broth tubes were plated on Modified Vogel Johnson agar (Buchanan et al., 1987) for confirmation of *L. monocytogenes*.

In order to choose appropriate heating times and temperatures for each sugar concentration or syrup, extensive small scale TDT tests were conducted for each sucrose solution or chocolate syrup. These were followed by more complete larger-scale experiments. The TDT data reported for *S. typhimurium* were based on results of TDT tests consisting of six different time intervals at each of 5 temperatures ranging from 65.6°C to 76.7°C. The TDT data reported for *L. monocytogenes* were based on TDT tests consisting of eight different time intervals at each of four temperatures from 60°C to 68.3°C.

The temperature data collected at the time of heating were analyzed using the general method (Stumbo, 1973) to establish cumulative lethality for each heating time interval. The general method formula used to establish cumulative lethality is:

$$L = \log^{-1}\left(\frac{T - T_R}{z}\right)$$

where L = cumulative lethality in minutes, T = measured temperature, T_R = reference (targeted test) temperature, z = the number of Fahrenheit degrees for a thermal death time curve to traverse one log cycle (negative reciprocal of slope of the thermal death time curve). This formula results in the calculation of an accurate lethality value for the entire time interval, including come-up-time. This corrected time or lethality was used in Stumbo's formula to calculate the decimal reduction (D) value at each time interval with surviving organisms:

$$D = \frac{t}{\log A - \log B}$$

where t = corrected lethality in minutes (L in previous formula), A = initial microorganism concentration, B = number of surviving microorganisms [assuming one viable organism per tube, as per Stumbo (1973)]. The D values were then averaged for each temperature. A phantom TDT curve (semi-logarithmic plot of D value vs temperature) was drawn by linear regression and the slope of the curve was used to calculate a z value. Since cumulative lethality (L) is a function of the z value used in the general method formula, which is initially unknown, it was likely that this z value would not be the same as that derived from the regression plot of D values. Therefore, when the data were initially analyzed, an arbitrary z value based on past experience was chosen to calculate lethalities. After the resultant phantom TDT curve was drawn the resulting z value was compared with the initial (arbitrary) z value. If there was a difference the calculated z value was substituted for the arbitrary z value and the entire calculation process was repeated. This was continued until the starting z value and calculated z value were essentially the same.

Table 2—D and z values of S. typhimurium *ATCC 13311 in chocolate syrup*

Syrup sample	a_w	pH	$D_{65.6°C}$ (min)	z value (C°)
A	0.83	5.10	1.2	6.2
B	0.84	5.10	2.6	7.6
C	0.75	5.65	2.7	8.3
D	0.83	5.35	3.2	7.7

Table 3—D and z values of L. monocytogenes *Scott A in sucrose solutions of various water activities*

	D-value (minutes)				
	Temperature (°C)				z-value
a_w	60	62.8	65.6	68.3	(C°)
0.98	2.0	0.74	0.36	0.15	7.6
0.96	2.9	0.97	0.52	0.30	7.9
0.94	5.6	3.0	1.1	0.47	7.6
0.92	7.6	5.3	3.1	1.6	12.3
0.90	8.4	5.9	3.8	1.9	12.9

RESULTS & DISCUSSION

THE HEAT RESISTANCE of the *S. typhimurium* strain increased as sucrose concentration increased and water activity decreased to a value of 0.83 (Table 1). The $D_{65.6°C}$ was 0.29 min at a_w 0.98 and 40.2 min at a_w 0.83. This was consistent with results of other researchers who also reported that as water activity decreased, heat resistance of *Salmonella* increased (Gibson, 1973; Baird-Parker et al., 1970; Corry, 1974; Goepfert et al. 1970).

Baird-Parker et al. (1970) obtained higher heat resistance. Using heat resistant and heat sensitive strains of *S. senftenberg* and *S. bedford* heated in solutions of heart infusion broth with sucrose, they obtained D values at 60°C of 4 to 12.6 min at 0.98 a_w and 46.7 to 75.2 min at a_w 0.90. Gibson (1973) reported a $D_{65.6°C}$ of 0.83 min at an a_w of 0.94 (compared with 1.4 min in this study) and a $D_{65.6°C}$ of 3.53 min at an a_w of 0.85 (compared with 19.2 min reported here). Differences between the data of others and those reported here reflect differences in strains, as well as experimental conditions. Goepfert et al. (1970) reported that the response of *Salmonella* to decreasing a_w was not uniform among strains.

The z values from phantom TDT curves of *S. typhimurium* at each a_w level were found to be relatively constant, ranging between 6.5 and 7.7°C (Table 1). This is in contrast to the data of Gibson (1973), who reported z values of 14.5 to 17°C at a_w levels of 0.94 to 0.99 but a z value of 20°C at 0.90 and 23°C at 0.85 for *S. typhimurium*. *S. senftenberg* also showed an increase in z value at lower a_w levels, but the change was less marked (10.9–13.5°C at a_w 0.94–0.99, 16.5°C at a_w 0.90, and 19.7°C at a_w 0.85).

To compare data collected in the model heating menstruum to data generated using a food product, two thermal death time experiments were performed using each of four chocolate syrups as the heating menstruum (Table 2). *S. typhimurium* heated in syrup A had a relatively low $D_{65.6°C}$ of 1.2 min as compared to the other three syrups ($D_{65.6°C}$ = 2.6 - 3.2 min). That *Salmonella* strain was about 3 times more heat resistant in syrup D than syrup A. The highest $D_{65.6°C}$ value of *Salmonella* in chocolate syrup, 3.2 min, was considerably lower than the heat resistance demonstrated in the sugar solution of comparable water activity (Table 1). This probably reflected in part differences in composition of the syrups, particularly sweeteners. Goepfert et al. (1970) found that glycerol and fructose provided little protection to *Salmonella* compared with sucrose, and concluded that survival of *Salmonella* during heating was a function of composition rather than a_w of the environment. Although our data suggested that both composition and a_w affected heat resistance, without information on the composition of the syrups we could draw no conclusions. Combinations of pH, solute type, preservatives (type and amount) may in-

teract with heat and a_w to affect heat resistance of microorganisms.

The heat resistance of *L. monocytogenes* increased as sucrose concentration increased and a_w decreased (Table 3). These results were similar to the results for *S. typhimurium*, although the magnitude of the increase was much less. The heat resistance of *L. monocytogenes* appeared to be comparable to that of *S. typhimurium* at 65.6–68.3°C over the range of a_w values tested (Tables 1 and 3). The heat resistance of *L. monocytogenes* at 0.98 and 0.96 a_w was consistent with data obtained by other researchers when heating the same organism in milk. Donnelly and Briggs (1986) obtained D values at 62.7°C of 0.35–1.0 min. However, our values were somewhat higher than those of Bradshaw et al. (1985, 1987), who obtained D values at 63.3°C of 0.22–0.58 min and at 66.1°C of 0.10–0.29 min. Our values were also slightly higher than those reported by Donnelly et al. (1987), who obtained D values at 62.2°C of 0.1–0.4 min. At lower a_w levels (0.90–0.94) the heat resistance of *L. monocytogenes* was notably greater than that reported previously. Of particular note is that at a_w levels of 0.90–0.92 the z value increased significantly, resulting in greater survival at higher temperatures than might be expected from extrapolation of data from higher a_w levels.

CONCLUSIONS

THE HEAT RESISTANCE of *S. typhimurium* and *L. monocytogenes* increased as sucrose concentration increased and a_w decreased. D values for *S. typhimurium* in chocolate syrup were 10-fold less than those for *S. typhimurium* in sucrose solutions of the same a_w but higher than those at a_w values 0.98 and 0.94. Thus a heat process for chocolate syrups based on heat resistance of *S. typhimurium* in sucrose solutions of comparable a_w would be conservative. The D values obtained for *L. monocytogenes* did not increase with decreasing a_w as much as did D values for *S. typhimurium*. Nevertheless, when determining processes for products with reduced a_w the increased heat resistance of *L. monocytogenes* at lower a_w must be considered. The increase in z value shown by *L. monocytogenes* at lower a_w values must also be considered, as heat resistance at higher temperatures would be greater than observed here for *S. typhimurium*. Also note that when performing TDT tests on reduced a_w substrates the effects of changing the osmotic environment must be considered when establishing the experimental protocol.

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Rheology of Sol-Gel Thermal Transition in Cowpea Flour and Starch Slurry

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ABSTRACT

A thermal scanning rigidity monitor was used to follow rheological changes during heating of cowpea flour and starch slurries. The gelantinization temperature of cowpea starch was in the range 67–78°C. For cowpea flour, in addition to starch gelatinization, a shallow plateau was observed. The starch gelatinization onset temperature shifted from 67°C for starch to 72°C for 25% cowpea flour that contained 12–15% starch. The modulus (G') of cowpea gels increased with flour concentration according to a power relationship. Rigidity of the cowpea starch and flour gels decreased at temperatures higher than 78 and 87°C, respectively.

Key Words: cowpea, flour, slurry, sol-gel, thermal-transition, rheology

INTRODUCTION

PROTEIN and starch slurries are known to exhibit drastic changes in flow and deformation properties on transformation into gels. These changes which may be induced by heat are often referred to as thermal transitions (Hamann, 1987; Lund, 1984; Wu et al., 1985a,b; Montejano et al., 1983; Patana-Anaeke and Foegeding, 1985; Kim et al., 1986). A continuous nondestructive procedure using small strain rigidity scan is one of the methods employed in probing the transition as the slurry traverses the range of processing temperatures. The information sought includes the transition temperature, transition characteristics as well as factors influencing gelation. This information is useful in the design and evaluation of gel texture.

Hamann (1987) observed that although small strain gel rigidity estimates did not correlate well with sensory texture or rupture strength, the procedure was adequate for monitoring physical property changes in the gel that related to molecular changes. Changes in gel rigidity or elasticity provide circumstantial evidence regarding structural changes in the gel during a recorded time and/or temperature increment. These changes may be related to protein unfolding, bonding of molecules, and starch gelatinization. Changes occurring during heat-induced gelatinization of starches have been examined by several researchers including Eliasson (1986), Osorio and Steffe (1987), Hansen et al. (1990), and Evans and Haisman (1979).

The objectives of our study were: to determine the flow and deformation properties during the sol-gel transformation of cowpea starch and flour slurries, and to relate the results to the firmness profiles of cowpea gels produced by thermal processing.

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MATERIALS & METHODS

Conditioning and milling of cowpea

Cowpeas were conditioned, decorticated, and milled as described in detail elsewhere (Okechukwu et al., 1991).

Preparation of cowpea slurries and gels

Weighed amounts of cowpea flour, salt and peanut oil were added together and manually mixed using a plastic ladle with sufficient water to give desired concentrations of cowpea slurry that was held with intermittent agitation for 30–60 min at 70°C. To ensure thorough and even dispersion of the constituents, the slurry was passed through an 850 micron sieve and oversize lumps were reduced and remixed with the main underflow slurry. After mixing, the slurry was left to stand for 1 hr to allow for adequate hydration; this also resulted in a lower slurry temperature. The cowpea slurries were sealed in 307 × 409 cans with 1.1 cm head space and heated in a stationary retort at 100°C for either 60 or 120 min to form gels.

Cowpea starch extraction procedure

The methods described by Cheng-Yi and Shin-ming (1981) and Schoch and Maywald (1968) provided the basis for extraction of starch from cowpea flour. At a liquid to solids ratio of 5:1, cowpea flour was mixed with 0.2% sodium sulfite and allowed to stand for 2 hr at about 20°C. The slurry was filtered through an ASTM No. 140 standard sieve (Newark Wire Cloth Co., Newark, NJ) with pore size 106 microns. The filtrate was collected and allowed to stand undisturbed for about 2 hr to allow the prime starch to sediment. The supernatant was decanted and the sediment reslurried with distilled water and allowed to sediment. The prime starch was washed (reslurried and allowed to settle) 3 times and finally collected in a stainless steel tray for drying in a vacuum oven at 35°C for 48 hr.

Dynamic rheological test

A tube and plunger Thermal Scanning Rigidity Monitor (TSRM) shown in Fig. 1 was used for dynamic tests that were designed to assess the rheological changes that occur in cowpea and starch slurries as temperatures gradually increased from ambient to 90°C. The TSRM consisted of a jacketed brass tube of internal diameter 20.1 mm and a brass plunger of external diameter 17.8 mm. The lower portion of the tube was covered with a Teflon plug bearing a type T thermocouple which was connected to a digital temperature display (Omega Engineering Inc., Stamford, CT). The Teflon provided a rigid support for the column of slurry in the annulus as well as insulation against heat loss. The plunger had two internal axial ducts for circulating a heating liquid and promoting heat transfer to the thin annular column of slurry.

The plunger was attached to a B load cell of the Universal Testing Machine (UTM), (Instron Engineering Corp., Canton, MA) and gradually lowered into the tube leaving a clearance of 2 mm above the Teflon plug. About 8 mL of slurry was injected into the annular space of the TSRM and gradually heated by circulating heating water from a variable temperature bath (Lauda K-2/R, Brinkman Instruments, Westbury, NY) through the jacket of the tube and the ducts of the plunger. The UTM was switched into an oscillatory mode with a cross head speed of 0.05 cm/min, plunger displacement of 0.1 mm, and a chart speed of 5 cm/min. The time required to attain each integer value of temperature of the gel was manually recorded starting from the moment the chart recorder was switched on. Heating rates during the tests ranged between 0.21–0.32°C/min. At the end of the test, the length of the plunger covered by the slurry was measured and used in estimating the apparent storage rigidity modulus, G'.

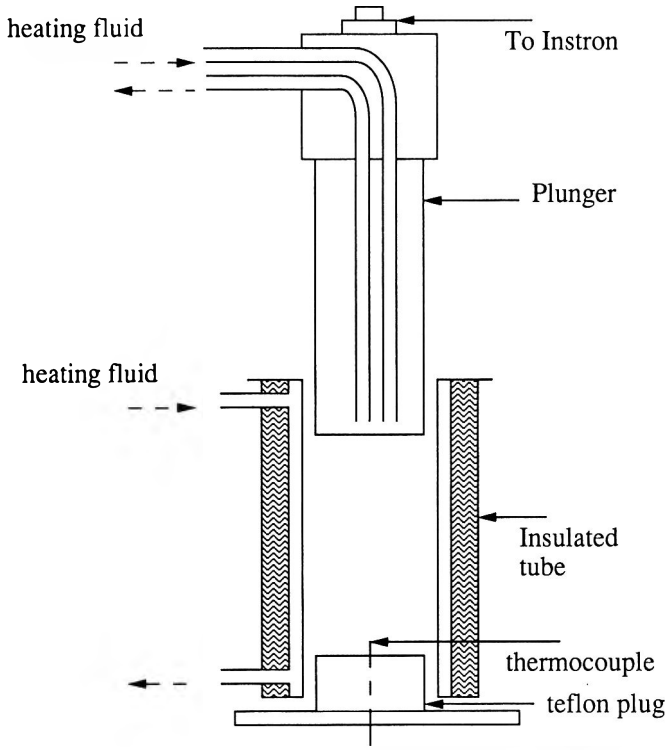

Fig. 1—Tube and plunger Thermal Scanning Rigidity Monitor (TSRM).

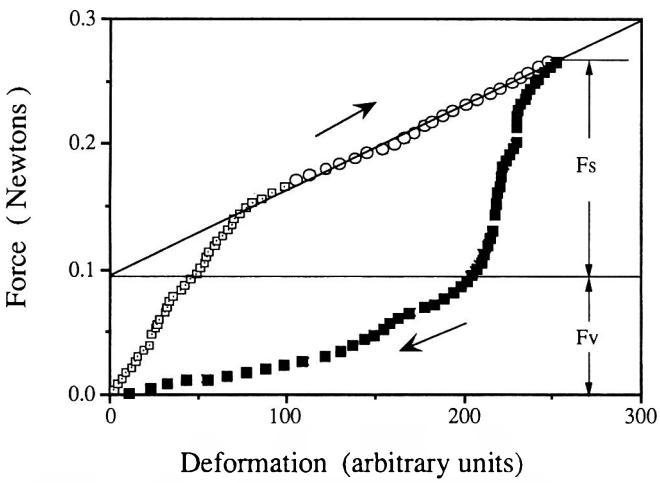

Fig. 2—Dynamic force-deformation relationship for 25% cowpea slurry at 52°C. (Arrow shows direction of plunger movement. F_s elastic force; F_v viscous force.)

Values of force as a function of plunger displacement for chosen cycles were obtained from the chart by using a digitizer (Summagraphics Corp., Fairfield, CT) connected to a Macintosh Plus computer (Apple computer Inc., Cupertino, CA). Every oscillation of the initial portions of the force-time record was digitized, but later portions of the oscillations for digitizing were chosen at intervals of 1-2 min. However, cycles which showed considerable base line drift were ignored. For each oscillation digitized, the force was plotted vs. plunger displacement and delineated for estimates of the storage (F_s) and viscous force (F_v) components of the peak force (Fig. 2) that are considered typical components of a Kelvin viscoelastic model. Both the viscous force and the spring (solid) force are forced to move together at the start of deformation. However, the viscous force attains an instataneous constant value F_v (Mohsenin, 1986). Beyond this limiting value F_v, the force deformation response is entirely a reflection of the solid behavior. The areas under the force-displacement curves were numerically estimated (IMSL, Houston, TX). The storage force and the calculated areas were used to evaluate the storage rigidity modulus, G', and the energy loss, respectively, according to the method outlined by Wu et al. (1985a) and Hamann (1987).

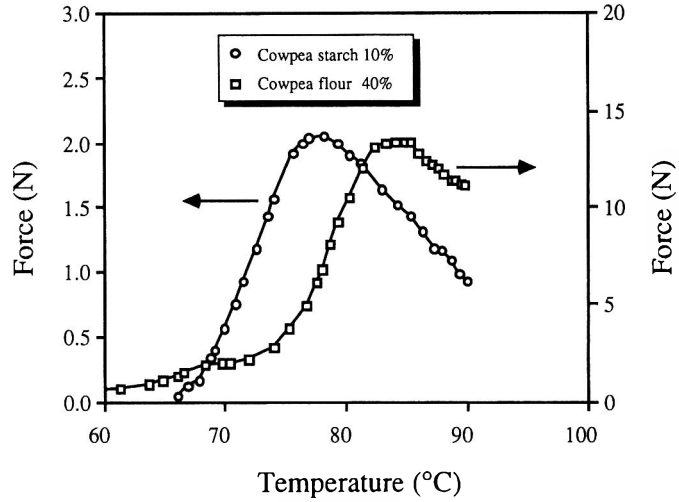

Fig. 3—Variation of peak force with temperature during thermal transition of 10% cowpea starch and 40% cowpea flour using the Instron in dynamic mode.

The rigidity modulus, G', defined as the ratio of shearing stress and elastic shearing strain (Mohsenin, 1986) can be evaluated for a thin section of gel of length L, at radial distance r, within the annular region of the TSRM. Over a small distance dr, G' can be expressed in terms of Eq. (1)

$$\frac{F_s}{2\pi Lr} = -G'\frac{dD}{dr} \quad (1)$$

where D is the plunger displacement and L is the length of the plunger covered by the slurry. Equation (1) can be solved for the physical boundaries of shear given in Eq. (2) to give Eq. (3).

$$\text{at} \quad r = R_1, \text{ deformation } (D) = D_1 \text{ and at} \quad r = R_2, D = 0 \quad (2)$$

where, R_1 and R_2 are the radii of the plunger and tube, respectively,

$$G' = \frac{F_s}{2\pi LD} \ln\frac{R_2}{R_1} \quad (3)$$

To compensate for the additional compressive force experienced by the plunger at the flat surface, Wu et al. (1985b) and Hamann (1987) modified Eq. (3) with an additional term given by Smith et al. (1949). This modified expression was used in evaluating G':

$$G' = \frac{F_s}{2\pi LD_1}\left(\ln\frac{R_2}{R_1} - \frac{R_2^2 - R_1^2}{R_2^2 + R_1^2}\right) \quad (4)$$

The % energy loss for each cycle ($\%E_{loss}$), defined as the percentage of the total energy input dissipated by viscous heating, was evaluated using Eq. (5):

$$E_{loss} = \frac{Area_1 - Area_2}{Area_1} \times 100 \quad (5)$$

where $Area_1$ and $Area_2$ are the areas under the force deformation plots for the positive and negative displacements of the plunger for a cycle, respectively.

Firmness of cowpea gels. Firmness of cylindrical sections of the cowpea gel was determined by the puncture test using the Voland Texture Analyzer (Voland Corp., Hawthorne, NY) equipped with an aluminum probe of diameter 6.3 mm. Force deformation response was recorded on a strip-chart recorder (Honeywell Electronik 196). Firmness was measured as hardness 1 and rupture slope as described elsewhere (Rao et al., 1989; Paulson and Tung, 1989).

RESULTS & DISCUSSION

Transition temperature of cowpea starch

Figure 3 illustrates the variation of peak force with deformation when a slurry of starch or cowpea flour was gradually

Table 1—Gelatinization and rigidity onset temperatures, and rigidity development rates for cowpea starch and flour slurries[a]

Material	Water/solids[b] ratio	Rigidity onset (°C)		T_c (°C)	Rigidity rate (Pa/°C)		Peak modulus[c] (Pa)
		Plateau	Starch		Plateau	Starch	
10% starch	9.0	–	69	78	–	2.7	32.3
25% flour	3.4	69	72	87	0.5	2.6	65.4
30% flour	2.7	67	73	87	0.7	10.0	91.3
35% flour	2.2	64	73	87	1.3	15.3	135.3
40% flour	1.8	54	73	86	1.3	14.3	147.7

[a] T_c is final gelatinization temperature.
[b] Water/solids ratios were based on dry weight of solids.
[c] Maximum value of rigidity modulus.

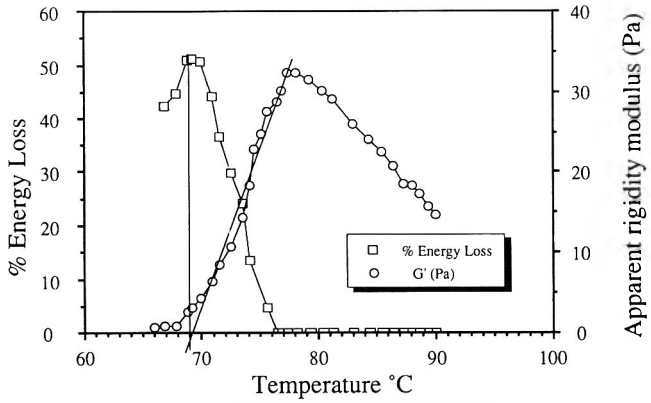

Fig. 4—Energy loss and apparent rigidity modulus in 10% cowpea starch thermal transition (—app. rigidity delineation).

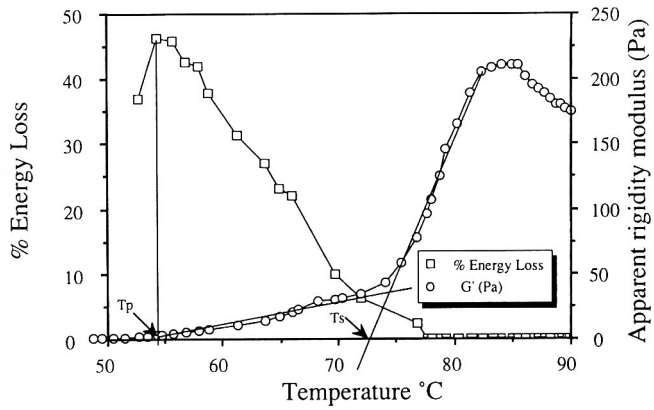

Fig. 5—Variation of apparent rigidity modulus and energy loss during the thermal transition of 40% cowpea slurry (Tp protein rigidity onset, Ts starch rigidity onset).

heated from about 40 to 90°C. Essentially, two segments which show an initial non-linear and a later linear relationship between force and deformation are apparent. Similar trends have been reported by Wu et al. (1985a) and Hamann (1987) for thermal transitions in proteins. The peak force, F_p, can be resolved into 2 components: a viscous force F_v and an elastic or solid force, F_s. The eastic force shows a linear increase with increase in deformation.

Figure 3 also shows an initial lag followed by a steep linear increase in force with temperature. The increase terminated in a peak force from where a decrease was observed with increase in temperature. The decrease in force beyond the peak may be related to a structural breakdown as reported for protein gels (Stanley, 1987) or may reflect a property decrease with temperature similar to temperature response on viscosity in the Brabender amylograph (Schoch and Maywald, 1968). Since the force was monitored throughout the temperature span of 40–90°C, the force changes should indicate heat-induced transitions such as gelatinization within the span.

From Figure 3, the gelatinization temperature range for cowpea starch was estimated as 67–78°C. Although the data were obtained for 10% starch slurry, the trends of the results would be applicable for other lower cowpea starch concentrations because the transition temperatures for starch are not significantly affected by concentration at high water/starch ratios (Lund, 1984; Wu et al., 1985a). This gelatinization temperature range compared favorably with estimates of about 66–77°C for starches from navy beans at low concentration using the Kofler hot stage microscopic method and had about the 10° temperature range for most starches (Lund, 1984).

The variation of peak force with temperature for 40% cowpea flour (Fig. 3) was typical of the trends observed also for 25, 30, and 35% cowpea flour slurries. Several important features distinguish the cowpea slurry thermograph from that of cowpea starch. In the cowpea thermograph the peak was replaced by a plateau which extends the gelatinization temperature range (Table 1). Because of the lack of additional data, such as with a differential scanning calorimeter, it is not possible to identify the component(s) responsible for the observed plateau. At the onset of starch gelatinization, the plateau contributed about 20% of the maximum force recorded during the gradual heating of the 40% flour slurry. There was a shift in the onset temperature of starch gelatinization (T_o) in the flour: 67°C for starch to about 72°C for 25% cowpea slurry that contained about 12-15% starch (Table 1). The onset gelatinization temperature also increased slightly with decrease in water/solids ratio for the cowpea dispersions (Table 1). This trend was consistent with the observed variation of T_o with moisture for rice starch (Lund, 1984) and it led to a slight decrease in the gelatinization temperature range with decrease in moisture. However, the final temperature, T_c, was apparently unaffected by variations in moisture within 25–40% cowpea concentration.

Rigidity development

Energy loss and apparent rigidity modulus (G') changed during thermal transition in 10% cowpea starch slurry (Fig. 4). The energy loss which measures the fraction of plunger energy lost as frictional dissipation (Rao, 1984) first increased from a measurable level of 42% to a maximum value of 51% at 69°C and then decreased to practically zero. The initial increase in energy loss was due to an increase in viscous dissipation resulting from viscosity decrease with increase in temperature. The decline in energy loss following the maximum value seemed to suggest the formation of elastic structures within the suspension and also was an indication of the rigidity onset temperature. The temperature at maximum energy loss coincided with the onset temperature indicated by delineation of apparent rigidity (Fig. 4).

Rigidity, measured by the apparent rigidity modulus, set in the starch dispersion at about 69°C, (a 2°C departure from the starch gelatinization onset, 67°C), increased sharply to a peak value at 78°C and decreased with further temperature rise. The decline in energy loss (energy damping) associated with the increase in rigidity modulus reflected the steady development of elastic structures in the course of starch gelatinization. The energy loss attained a near zero value very near to the tem-

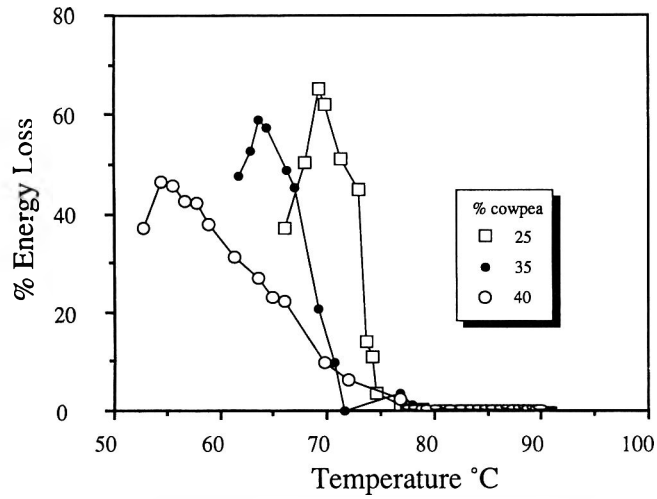

Fig. 6—Energy loss changes with temperature of 25, 35, and 40% cowpea flour gels.

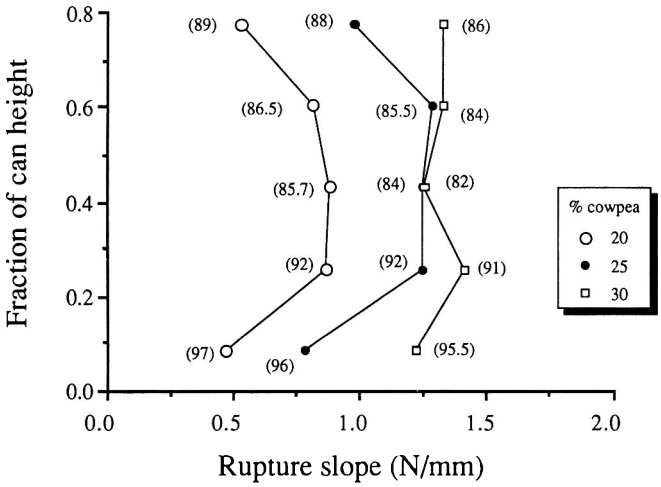

Fig. 7—Axial variation of firmness in 20, 25, and 30% cowpea gels processed at 100°C in vertical 307×409 cans for 60 min; ( ) measured temperatures in °C.

perature at peak rigidity. The attainment of almost 90% of the maximum gel rigidity at zero energy loss suggested that elastic development was associated with development of rigidity in cowpea starch gels.

The variation of apparent rigidity modulus and energy loss during the heating of 40% cowpea slurry is presented in Fig 5. The temperature at maximum energy loss coincided with the initial rigidity onset temperature. The development of rigid structures in the cowpea flour gel was seen to follow essentially a 2-stage process starting gradually from a rigidity onset temperature, lower than the onset temperature for cowpea starch. This slow initial step was followed by a steep phase of rigidity development that began at a temperature near the starch gelatinization temperature. While it is difficult to identify components responsible for the first stage without additional data, the second stage was obviously due to starch gelatinization. This trend in variation of rigidity modulus for 40% cowpea slurry was also observed for 35, 30, and 25% cowpea dispersions.

The rates of structural development with respect to temperature (Pa/°C) in both the initial plateau and starch gelatinization stages (Table 1) increased with increase in cowpea concentration. However, the rate for the 40% cowpea appeared to be inhibited and may be indicative of shortage of water necessary to meet the transition requirement. The rates during starch gelatinization were 5–12 times the rates during the initial plateau development for cowpea levels of 25–40%. Further examination of important features of the heat-induced transition revealed that the initial plateau development temperature decreased by 1.1°C for each percentage increase in cowpea solids from 69°C at 22.5% solids to 54°C at 36% solids. Although there was a shift from 69°C for pure starch to about 73°C for cowpea flour, there was no substantial difference in rigidity onset for the starch gelatinization stage of the thermal transition for the cowpea slurries.

Starch gelatinization temperatures are known to increase with large increases in starch/water ratio (Lund, 1984; Wu et al., 1985b). This phenomenon may have been responsible for the shift in start rigidity onset temperature as the starch/water ratio was increased. This information is particularly useful in process design intended to increase slurry viscosity to minimize settling and phase separation during heating when slurry energy loss was on the increase. Some rigidity sufficiently high to maintain uniform consistency within the slurry could be thermally induced by holding a cold slurry at temperatures just below the starch rigidity onset with occasional agitation. This may be the basis for the method of Ngoddy et al. (1986) in which moinmoin slurry was made by mixing all ingredients with hot water at 70°C.

Figure 6 shows the variation of energy loss with temperature for 25, 35, and 40% cowpea slurried during gel transformation. The energy loss increased initially to a maximum then fell rapidly and later at a slow rate to almost zero at about 78°C. Because the elasticity was at a maximum when the energy loss was zero, there was an almost complete transformation to an elastic gel at 78°C. Estimates on the apparent rigidity modulus show that at that temperature, 70, 55, 50, and 46% of the peak rigidity was developed in the 25, 30, 35, and 40% cowpea flour gels, respectively. For the cowpea starch gel with almost zero energy loss at 78°C, about 90% of the peak rigidity was attained at maximum elasticity. Substantial increase in rigidity was, therefore, seen for the cowpea flour gels beyond the attainment of maximum elasticity as shown in Fig. 5 for 40% cowpea concentration. This further strengthening of elastic structures in the gel may be attributed to interactions with the cowpea proteins. Similar protein starch interactions have been reported previously by Wu et al. (1985b) on fish actomyosin mixed with starch.

Rigidity variation with cowpea flour concentration

The peak rigidity modulus (G′) increased with increase in the % cowpea solids (C) in the gel (Table 1). Correlation of the data points in order to establish the concentration dependency suggested linear, quadratic, exponential and logarithmic relationships as possibilities. Equations (6) through (9) show that the four possibilities had good R^2 values. An expression similar to Eq. (8) was reported for the concentration dependence of the rigidity modulus of corn starch gel on cooling (Hansen et al., 1990).

$$G' = 6.5C - 80.4 \qquad R^2 = 0.957 \quad (6)$$

$$G' = -191.7 + 14.3C - 0.13C^2 \qquad R^2 = 0.964 \quad (7)$$

$$\text{Log } G' = 1.21 + 2.75 \times 10^{-2}C \qquad R^2 = 0.949 \quad (8)$$

$$\ln G' = -1.52 + 1.84 (\ln C) \qquad R^2 = 0.966 \quad (9)$$

Rigidity decrease

The apparent rigidity modulus of cowpea starch gel (Fig. 4) decreased with increasing temperature from 78°C. Similarly a decrease in rigidity of cowpea gels, called terminal decrease or weakening, was recorded (Fig. 5) for temperatures higher than 87°C. These trends were similar to the terminal decrease in elastic modulus observed during the thermal transition of potato and waxy barley starch suspensions by Eliasson (1986).

A reduction in the magnitude of rigidity following the first transition peak was also observed for corn starch by Osorio and Steffe (1987) and for wheat and maize starches by Eliasson (1986). Viscosity, another viscoelastic property, is also known to decrease for most starches beyond the peak in visco-amylograph studies (Wu et al., 1985a; Schoch and Maywald, 1968; Deffenbaugh and Walker, 1989; Varriano-Marston et al., 1980).

Terminal weakening of elastic structures in protein gels was reported by Hamann (1987), Kim et al. (1986), Wu et al. (1985a,b), and Montejano et al. (1983) at temperatures higher than 60°C. This weakness in fish protein gels has been attributed by Kim et al. (1986) to a thermal effect of proteolytic activity. In soy protein gels terminal weakening of gel structure observed at temperatures above 80°C was traceable to damage of the gel microstructure (Furukawa et al., 1979; Stanley, 1987). Although the exact cause and mechanism of structural weakness in starch or starch/protein gels such as cowpea gel is not known, 2 factors may be responsible: (1) a temperature dependence in which the rigidity modulus decreases with increase in temperature (Hansen et al., 1990) in a functional form similar to Arrhenius dependency of gel viscosity on temperature (Doublier, 1981; Dolan and Steffe, 1989), and (2) a thermal damage reflecting over-processing and traceable to alterations in gel microstructure as found for soy gels by Furukawa et al. (1979). While the first is a physical phenomenon and would produce reversible structural changes, the second would produce irreversible changes which should be manifested in the gel after it is cooled.

Processing implications

Studies by Paulson and Tung (1989) on protein gels showed that the storage modulus (G') correlated well with rupture slope from puncture tests. Moin-moin gels are obtained from slurries containing about 15% cowpea solids or more that are processed in steam or boiling water in small metallic containers or leaf pouches. Temperature distribution within the gel results from thermal conduction during the process of gelation (Okechukwu et al., 1991). In light of the variation of storage modulus with temperature, textural variations in cowpea gels are expected to reflect the distribution of maximum temperature attained in the course of gelation.

Figure 7 shows the axial variation of rupture slope for 20, 25 and 30% cowpea slurries containing 8% vegetable oil and 1% salt processed into gels at 100°C in upright 307 × 409 cans for 60 min. In parenthesis on the curves are measured temperatures (°C), just before the cooling cycle, at the indicated axial positions in the cans. Generally, there was a decrease in firmness toward the top and bottom regions of the can where maximum temperatures were all higher than 87°C. The profile for the 30% slurry showed no loss of firmness towards the top of the can where temperatures were within the range for rigidity development. However, there was a depression in firmness at about the can center due to the low temperature (< 87°C) at that section of the can. Firmness profiles for the same concentrations of cowpea slurries after 120 min of processing at 100°C (not shown here) also showed decrease in firmness toward the high temperature regions of the top and bottom of the cans and also shifted in the direction of lower rupture slope after processing for 2 hr at 100°C. The trends exhibited by radial firmness profiles (not shown here) were similar to those of the axial profiles. As expected, the maximum firmness was at the gel center where heating was minimal.

CONCLUSION

THE TSRM provided useful data on thermal transitions during the heating of cowpea starch and flour slurries, such as starch gelatinization onset and final temperatures. The gelatinization onset temperature increased with decrease in water/solids ratio of the cowpea slurries. The increase in peak rigidity modulus with % cowpea solids could be described best by exponential and power relationships. The results with TSRM helped explain observed variation in firmness of thermally processed cowpea gels.

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A Research Note

Production of Extracellular β-Glucosidase and α-Galactosidase during Fungal Growth on Polygalacturonate

A.M. McKAY

ABSTRACT

Filamentous fungi (*Aspergillus oryzae, Scopulariopsis* sp., and *Penicillium brevicompactum*) and yeast-like fungi (*Aureobasidium pullulans*) were induced to produce extracellular β-D-glucosidase (β-D-glucosidase glucohydrolase, EC 3.2.1.21) and α-D-galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22) by polygalacturonate in the growth medium. These extracellular enzymes were not produced during growth of the microorganisms on cellobiose or melibiose.

Key Words: glucosidase, galactosidase, glucohydrolase, polygalacturonate, enzymes, microbes

INTRODUCTION

FILAMENTOUS FUNGI grown on bran-based medium in semi-solid culture produce a wide range of extracellular enzymes which may be used in biotechnology. Enzymes for use in the food industry are most commonly produced by *Aspergillus* spp. and include amylases, proteases, lipases, pectic enzymes and β-galactosidases. Aspergilli can also produce extracellular α-galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22) (Cruz and Park, 1982; Annunziato et al., 1986) and β-glucosidase (β-D-glucoside glucohydrolase, EC 3.2.1.21) (Wase et al., 1985; Yeoh et al., 1986). Extracellular β-glucosidase enzymes are also produced by *Penicillium* spp. (Parr, 1983; Brown et al., 1987). During growth in submerged culture in a defined medium extracellular β-galactosidase production by *Aspergillus oryzae* and *Scopulariopsis* sp. only occurred with polygalacturonate as substrate (McKay, 1991). Extracellular β-galactosidase production is also associated with growth on polygalacturonate by *Penicillium brevicompactum* and by the yeast-like fungus *Aureobasidium pullulans* (unpublished results).

Polygalacturonate is a complex carbohydrate consisting of a central polymer of galacturonic acid residues interspersed with rhamnose residues to which sidechains of neutral sugars, mainly galactose, arabinose and xylose, are attached (Rombouts and Pilnik, 1980). During degradation of polygalacturonate, filamentous fungi may produce a complex mixture of oligosaccharides, which may induce secretion of extracellular saccharolytic enzymes.

Our objective was to investigate the production of extracellular α-galactosidase and glucosidase enzymes during growth of filamentous fungi and the yeast-like fungus *Aureobasidium pullulans* on polygalacturonate in submerged culture.

MATERIALS & METHODS

ASPERGILLUS ORYZAE strain 20423 and *Scopulariopsis* sp strain 44206 were supplied by the American Type Culture Collection (12301 Parklawn Drive, Rockville, MD 20852). *Penicillium brevicompactum* and *Aureobasidium pullulans* strains were isolated from soil samples and maintained in the culture collection of the Food Microbiology Department (Newforge Lane, Belfast). The isolated microorganisms were maintained on malt extract agar slants. The fungi were grown in submerged culture in Erlenmeyer flasks shaken at 22°C for 4 days. The growth medium consisted of yeast nitrogen base, potassium phosphate pH 5 (final concentration 100 mM) and 1% potassium polygalacturonate (Sigma Chemical Co Ltd).

Cultures were centrifuged at 14,000 rpm in an Eppendorf microfuge. Supernatants were assayed using the chromogenic substrates, p-nitrophenyl α-galactoside, p-nitrophenyl α-glucoside and o-nitrophenyl β glucoside, supplied by Sigma Chemical Co Ltd.

The assay mix contained chromogenic substrate (200 μL, 50 mM stock solution) and potassium phosphate pH 5 (100 mM final concentration). Reaction mixes were equilibrated at temperature for 10 min and started by addition of enzymes (20–150 μL) to give a 1 mL reaction volume. The reaction was stopped by addition of 1M sodium carbonate pH 11.4, (0.5 mL). Liberated p-nitrophenol was estimated at 405 nm (extinction coefficient 1.83×10^4 M^{-1} cm^{-1}) or o-nitrophenol at 420 nm (extinction coefficient 4.5×10^3 M^{-1} cm^{-1}) (Dickson and Markin, 1980; Annunziato et al., 1986).

RESULTS & DISCUSSION

NONE OF THE FUNGI produced extracellular α-glucosidase activity during growth on polygalacturonate. The fungi produced extracellular α-galactosidase and β-glucosidase enzymes with temperature optima in the range 50–65°C (Fig. 1). Activity of β-glucosidase enzyme during growth on polygalacturonate was higher than α-galactosidase activity for all fungi tested (Table 1).

Extracellular β-glucosidase and α-galactosidase enzymes were not detected during growth of these fungi on cellobiose or melibiose in submerged culture. A similar failure to induce an enzyme by addition of disaccharide has been shown for the extracellular β-galactosidase of *Aspergillus oryzae* and *Scopulariopsis* sp. by lactose in the growth medium (Park et al., 1979; Pastore and Park, 1979; McKay, 1991). Thus the growth of fungi on the complex carbohydrate polygalacturonate can result in induction of secretion of extracellular galactosidase and glucosidase enzymes. These enzymes may participate in hydrolysis of bonds between sugar residues other than the galacturonic residues in the polymer. The results may have application in the commercial production of extracellular galactosidase or glucosidase enzymes. In addition pectic enzymes from filamentous fungi are widely used in the food industry and the presence of galactosidase and glucosidase enzymes in such preparations may have important secondary effects in food products.

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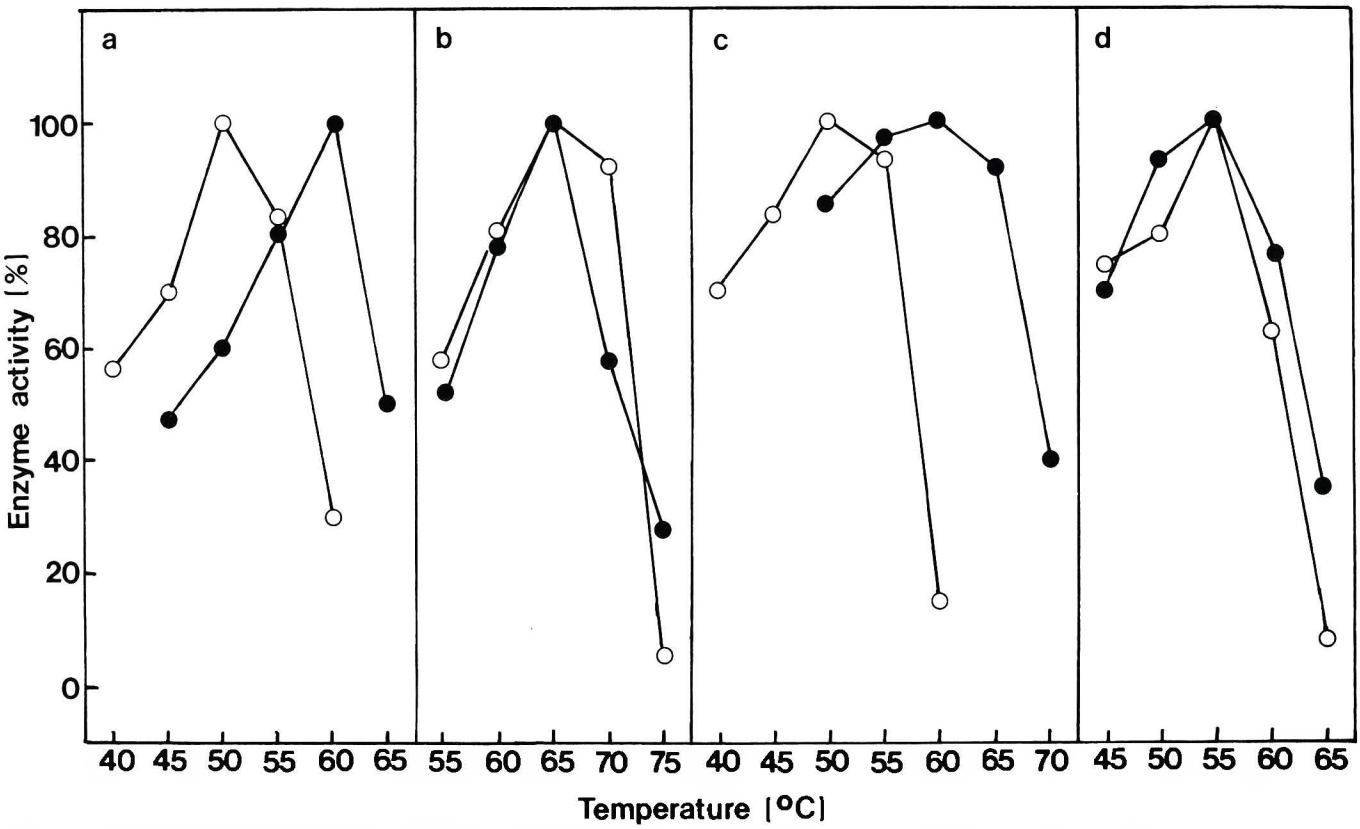

Fig. 1—Effect of temperature on β-glucosidase (●———●) and α-galactosidase (○———○) activity: (a) Aspergillus oryzae*; (b)* Scopulariopsis *sp.; (c)* Penicillium brevicompactum*; (d)* Aureobasidium pullulans.

Table 1—Extracellular α-galactosidase and β-glucosidase yields for fungal species growing on polygalacturonate

Microorganism	Enzymes activity[a] (n mole nitrophenol min^{-1} mL^{-1}) α-galactosidase	β-glucosidase
Aspergillus oryzae	138 ± 14 (50°C)	219 ± 17 (60°C)
Scopulariopsis sp.	23 ± 1 (65°C)	56 ± 7 (65°C)
Penicillium brevicompactum	4 ± 0.5 (50°C)	46 ± 4 (60°C)
Aureobasidium pullulans	48 ± 17 (55°C)	100 ± 15 (55°C)

[a] Enzyme activities determined at temperatures in parentheses. Results are means of 3 samples with standard deviations of the mean.

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A Research Note

In-Vitro Protein Digestibility of Dehydrated Protein Extract from Poultry Bone Residue

J.O. OPIACHA, M.G. MAST, and J.H. MacNEIL

ABSTRACT

In-vitro protein digestibility of freeze- or spray-dried protein obtained from poultry bone residue by alkali extraction was evaluated by a multienzyme procedure. Digestibility of the dehydrated extracts was significantly lower than chicken breast muscle or mechanically deboned poultry. Digestibility of the spray-dried extracts was about 3% higher than freeze-dried extracts.

INTRODUCTION

PROCESSES with mild alkaline or salt solutions have been used to recover residual meat protein from bone residue and trimmings resulting from poultry and red meat mechanical deboning operations (Young, 1976; Hamilton, 1978; Golan and Jelen, 1979; Jelen et al., 1979; Palka et al., 1985; Lawrence et al., 1982; Kijowski and Niewiarowicz, 1985). In most such processes, especially alkali extraction, protein is recovered as a "meaty" paste by precipitation with HCl. Salt extracts have been recovered either as liquid extracts or as dehydrated proteins salted-out by ionic strength reduction from the liquid extract.

Nutritional evaluation of the extracts has focused on protein recovered by acid precipitation. Golan and Jelen (1979) reported that the percent essential amino acids and protein efficiency ratio of alkali extracts of beef bones were comparable to beef. However, the essential amino acid index (EAAI) was lower due to the low tryptophan content of the extract. Similar results were obtained by Palka et al. (1985) using beef and pork residues. No differences in the EAAI were observed since the tryptophan content of the protein extract was similar to that of the meat. Ozimek et al. (1986) also indicated that the nutritional quality of alkali extracts of poultry bone residue was not substantially impaired by alkali extraction and acid precipitation as indicated by net protein utilization, protein efficiency ratio, true digestibility, and net growth studies. Limited information is available on the nutritional quality of the extracts in instances where protein would be recovered by direct dehydration of the liquid extracts. In our study, in-vitro digestibility of dehydrated protein extracted from poultry bone residue by alkali solubilization was evaluated.

MATERIALS & METHODS

POULTRY BONE RESIDUE, prepared from broiler backs and necks, was obtained frozen from a broiler processing plant. Protein was extracted from the bone residue by a procedure adapted from Golan and Jelen (1979) and Lawrence et al. (1982) as outlined in Fig. 1. The pH of the resulting liquid extract was reduced to 6.8–7.0 with 1M HCl for freeze-drying, and to 5.0–5.5 for spray-drying. Samples were freeze-dried in a Stokes freeze-drier Model 902-001-8 (Equipment Division, Pennsal Chemical Corp., Philadelphia, PA) at ambient temperatures (22–25°C) under 0.5mm Hg vacuum. Spray-drying was performed using a Niro Atomizer Portable Spray drier (Niro Atomizer, Copenhagen, Denmark) with evaporating capacity 16.8 kg/hr operated at a feed flow 30 mL/min (5% solids), inlet 175°C and outlet 85°C. The dried protein extracts were packaged in Ziploc bags (Dow Chemical Co., Midland, MI) and stored at −20°C up to 4 months.

In-vitro protein digestibility of the samples was determined by the multienzyme procedure of Hsu et al. (1977). Digestibility of the sample was compared to freeze-dried mechanically deboned poultry, chicken breast meat, and casein. Differences in digestibility were detected by analysis of variance and the means separated by the Duncan's (1955) multiple range procedure.

RESULTS & DISCUSSION

THE TWO DRIED EXTRACTS were significantly ($p<0.05$) less digestible than either chicken breast muscle or mechanically deboned poultry (Table 1). In addition, the spray-dried extract was more digestible than the freeze-dried sample. Since protein precipitated from alkali extracts of bone residue by HCl has similar nutritional quality to meat (Osimek, 1986; Palka et al. 1985), possibly some components which would otherwise be retained in the supernatant, e.g., heme proteins, changed during dehydration and storage. Also possibly differences in processing methodology were responsible for the lower di-

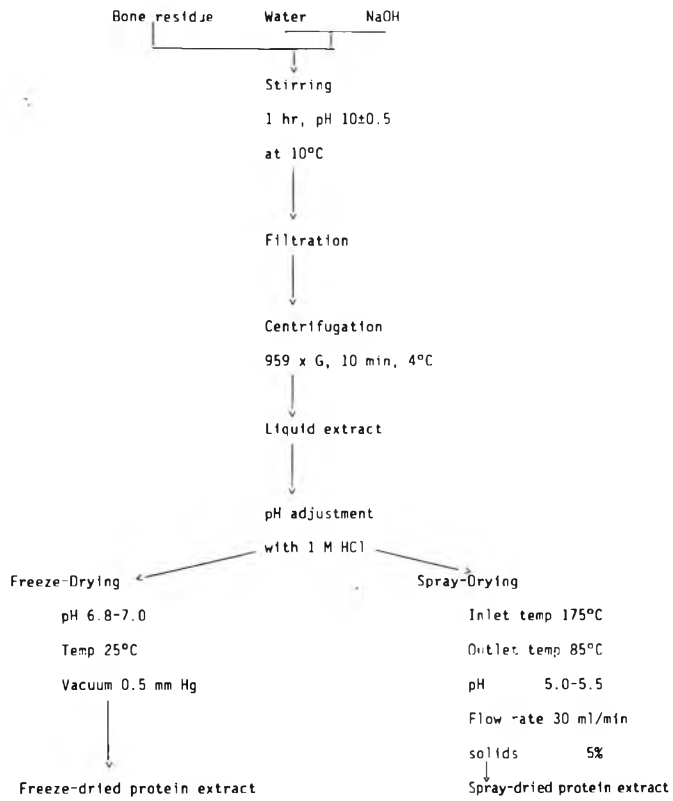

Fig. 1—Extraction and recovery of protein from poultry bone residue.

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Table 1—In vitro digestibility of poultry proteins

Sample	% Digestibility
ANRC[a] casein	93.5 ± 2.3 a[b]
Chicken breast meat[c]	89.3 ± 1.3 b
Mechanically deboned poultry[c]	89.1 ± 0.9 b
Spray-dried protein extract	87.3 ± 0.6 c
Freeze-dried protein extract	84.6 ± 1.8 d

[a] Animal Nutrition Research Council.

[b] Means of 7 determinations. Means with different letters are significantly different ($p < 0.05$).

[c] Unheated freeze-dried sample.

gestibility of the dehydrated protein extracts. Although de Groot (1963) observed no significant changes in protein quality due to dehydration in cooked, freeze-dried beef, fish and poultry, he indicated that dehydration at low temperatures, such as freeze-drying, could impair rehydration properties leading to reduced digestibility. Rehydration capacity is reduced due to protein-protein and protein-lipid interactions during storage. Maga et al. (1973) also indicated that heating could improve protein digestibility by promoting structural changes involving partial unfolding of polypeptide chains thus exposing susceptible bonds, and/or the release of lipid-bound proteins. Moderate heating did not significantly affect the nutritional value of proteins (Bender, 1960; Ledward, 1979). Therefore, if spray-drying would be an economical process for recovery of protein extracts of bone residue, loss of protein digestibility should not be a critical factor.

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• *J. Food Sci.* (1991) 56(5): 1302–1307. Thermal Processing of Cowpea Slurries by P.E. Okechukwu, M.A. Rao, P.O. Ngoddy, and K.H. McWatters. On page 1307, references, Okechukwu et al., 1991a, the correct journal citation should read: *J. Food Sci.* 56(6): 1744. Please change accordingly.

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