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IMPORTANT PUBLICATIONS & DEADLINES FOR . . . .

ift 85
45th Annual Meeting & Food Expo — June 9-12, 1985
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PUBLICATION	WHERE TO FIND IT	DEADLINES
Call for Symposia Proposals and Guidelines for Symposia Organizers	*Food Technology*, January 1984, pp. 8 & 154-160	Receipt of preliminary proposals: May 15, 1984. Notice of acceptance or rejection of preliminary proposals: July 15. Receipt of final proposals: October 1. Notice of acceptance or rejection of final proposals: November 15, 1984.
Call for Volunteered Papers and Official Abstract Forms	*Food Technology*, September 1984, pp. 97-106	Receipt of abstracts: December 1, 1984. Notice of acceptance or rejection of papers: February 1, 1984
Guidelines for Poster Presentations	*Food Technology*, September 1984, p. 96	Same as Call for Volunteered Papers
Rules Governing IFT's Graduate Paper Competitions	*Food Technology*, September 1984, pp. 94-95	Same as Call for Volunteered Papers
Call for Undergraduate Research Paper Competition Papers	*Food Technology*, September 1984, p. 95	Notify URPC chairman: January 15, 1985. Receipt of abstracts: February 15, 1985
Call for Films for Film Theater	*Food Technology*, September 1984, p. 93	Receipt of films: December 30, 1984
Call for Nominations for Achievement Awards and Fellows	*Food Technology*, September 1984, pp. 90-91	Receipt of nominations: Industrial Achievement Award, December 1, 1984 International Award, December 1, 1984 Appert Award, December 15, 1984 Cruess Award, December 15, 1984 Babcock-Hart Award, January 1, 1985 Prescott Award, January 1, 1985 Fellers Award, January 1, 1985 IFT Fellows, February 1, 1985
Call for Nominations for the new Donald K. Tressler Award	*Food Technology*, September 1984, pp. 92-93	Receipt of nominations: December 15, 1984
Preliminary Program and Registration Forms	Mailing date March 1, 1985. Program also published in *Food Technology*, May 1985	Receipt of advanced registration forms: May 15, 1985
IFT-IUFoST Basic Symposium, "Foodborne Microorganisms and their Toxins—Developing Methodology," to be held in Atlanta, Ga., on June 7-8, 1985	Program, abstracts, and registration forms: *Food Technology*, March 1985	Receipt of advance registration forms: May 15, 1985
IFT Short Course, Subject to be announced to be held in Atlanta, Ga., on June 12-14, 1985	Program and registration forms: *Food Technology*, March 1985	Receipt of advance registration forms: May 15, 1985
Food Expo Exhibit Prospectus	Mailing date August 29, 1985	Receipt of contracts: October 15, 1984
Official IFT Food Expo Information Form	Mailing date March 15, 1984	Receipt of forms: April 15, 1985
Official Listing of Exhibitors and Booth Numbers	*Food Technology*, May 1985	—
Resume Forms and Position Description Forms for Employment Bureau	*Food Technology*, March 1985	Receipt of forms: May 1, 1985
IFT 85 Program & Abstracts Booklets	Distributed to all registrants at Annual Meeting	—
IFT 85 Food Expo Directory of Exhibits	Distributed to all registrants at Annual Meeting	—
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Nutrient Composition of Lamb of Two Age Groups

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————————ABSTRACT————————

The nutrient composition of Spring and Fall lambs were investigated. Seven retail cuts from carcasses of lambs raised under commercial conditions, and representing two age groups (4–4½ mo and 8–9 mo) were analyzed in both raw and cooked form. Separable lean meat was analyzed for proximate composition, 8 vitamins, 8 inorganic nutrients, cholesterol and 12 fatty acids. Except for moisture, total lipid, riboflavin, niacin, Zn and Fe, there were no practical differences in nutrients between cuts or age groups. Thiamin had the lowest cooking retention with a range of 29.0–63.5%.

INTRODUCTION

IN 1981, 338 MILLION POUNDS (carcass weight equivalent) of lamb and mutton were produced in the United States and, during the same period, net import provided an additional 26 million pounds. Retail weight per capita was 1.41 pounds (USDA, 1981). Although this per capita figure might be useful for comparing it against consumption figures of other food items, it is a poor indicator of where lamb is consumed in the U.S. The major lamb consumption markets are the large cities in the Northeast. In 1978, the Northeast area accounted for about 60% of lamb and mutton consumed in the U.S. The New York Metropolitan Area alone accounted for 42% of the market (Eastern Lamb and Wool Marketing Conference, 1981). Thus, certain segments of our population are consuming significant amounts of lamb, and yet there is a lack of current and comprehensive nutrient composition data on lamb.

A classical study on the nutrient composition of cooked meat, including USDA Choice grade lamb, was conducted by Leverton and Odell (1959). In 1960, however, the USDA quality grade standards for lamb were revised and in 1969, USDA yield grade standards for lamb were introduced. These changes might have affected the type of lamb being produced. In 1960, the average live weight of slaughter lambs was 99 lb (USDA, 1972) but in 1980, the average was 110 lb (USDA, 1981). Leverton and Odell (1959) recognized that the scope of their study was limited and suggested that further studies be conducted at a later time.

Our study was initiated partly in response to that suggestion and also as part of a program to provide current and comprehensive data on red meats for the revision of USDA Handbook No. 8. For lamb, the current edition of Handbook No. 8 (Watt and Merrill, 1963) does not include any data which reflect the effects of the 1960 and 1969 changes in USDA grades and current production practices. Also, as noted by Watt and Murphy (1970) the 1963 edition of Handbook 8 lacks data on several minerals and vitamins. Kinsella and co-workers (1975), with reference to Handbook 8, pointed out the need for determining fatty acid

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content and composition of various retail cuts in order to confirm and expand the present data base which was extracted from random sources. Such comprehensive data will be valuable not only for the health scientists and consumers but also for the lamb producers who are interested in knowing how their production practices are affecting the nutrient composition of their product.

MATERIALS & METHODS

Lamb samples

Using the 1960 and 1969 USDA Quality and Yield Grades Standards, respectively, eight USDA Choice Grade (yield grade 1.80–3.75) lamb carcasses, ages 4–4½ months, were selected from lambs which were raised and slaughtered at Ohio State University. The lambs were Suffolk-sired from cross-bred ewes raised on shelled corn and SBM-mineral-vitamin pellets. The selection was limited to the Choice grade since over 80% of graded lamb falls into this category (USDA, 1983, personal communication). Carcasses were cut into wholesale cuts, vacuum packaged and shipped to the USDA Meat Science Research Laboratory (Beltsville, MD), for fabrication into retail cuts and processing for analysis. In order to assess the effect of age and changes in feeding regimen on the nutrient composition of retail lamb, eight USDA Choice Grade (yield grade 1.65–3.25) lamb carcasses, ages 8–9 months, were selected from the same flock from which the younger lambs had originated. The older lambs were grazed on pasture, supplemented with shelled corn and SBM-mineral-vitamin pellets. The two age groups simulated the major age groups of lambs which are marketed. Wholesale cuts were again shipped to Beltsville for processing.

Retail cut fabrication

Retail cuts not to exceed ½-inch of subcutaneous fat were fabricated from contralateral sides of each carcass in order to obtain matched pairs of cuts for processing as raw or cooked samples. The retail cuts included fore shank, arm chop, blade chop, rib roast, loin chop, sirloin half of leg and shank half of leg. All chops were cut to ¾-inch thickness. Fabrication into retail cuts were performed according to specifications described in the Uniform Retail Meat Identity Standards (National Live Stock and Meat Borad, 1973).

Cooking methods

One cut from each pair of cuts was cooked. The fore shanks and chops were braised. Fore shanks were each cooked in 1500 mL water for 1.5 hr in an oven set at 164°C. Arm chops were browned for 3 min on each side, and then cooked in 150 mL H_2O for 45 min at 164°C. Blade chops and loin chops were broiled on Farberware grills to an internal temperature of 76°C. Rib roasts, sirloin-half of leg and shank-half of leg were roasted in a conventional oven at 164°C to an internal temperature of 76°C. All cooked samples were weighed immediately after cooking and cooled to 50°C before obtaining cooled weights. The National Live Stock and Meat Board participated in the carcass selection and provided procedures and guidance in retail cut fabrication and cooking.

Dissection and homogenization

All samples, raw and cooked, were dissected into subcutaneous fat, intermuscular (seam) fat, lean, connective tissue and bone. With the exception of the lean from the fore shanks which required pooling of lean from two carcasses in order to have enough sample for analyses, the lean from each retail cut was weighed and then homogenized in a R-6 Robot-Coupe food processor set at 1800 rpm for 12 sec and 3000 rpm for 18 sec. Samples for moisture analysis were

Table 1—Nutrient values of raw separable lean from retail cuts of lamb

Nutrient	Age group[f]	Fore-shank[g]	Arm chop	Blade chop	Rib roast	Loin chop	Leg-sirloin	Leg shank
		Nutrient values (mg/100g lean, except where noted)[abcde]						
Moisture (g/100g)	A	75.29 (0.37)[h]	75.04ab (0.40)	73.31c (0.53)	71.16d (0.64)	73.10c (0.46)	72.98bc (1.76)	75.14a (0.30)
	B	75.23 (0.59)	74.26ab (0.36)	73.47c (0.18)	72.04d (0.45)	73.29c (0.36)	74.62ab (0.25)	75.14a (0.31)
Protein (g/100g)	A	21.30 (0.25)	20.64 (0.53)	19.90 (0.27)	20.26 (0.21)	21.35 (0.25)	20.44 (0.21)	21.06 (0.22)
	B	20.64 (0.97)	19.02 (0.19)	18.72 (0.14)	19.76 (0.25)	20.30 (0.08)	21.04 (1.28)	20.06 (0.12)
Total Fat (g/100g)	A	3.61 (0.25)	5.49c (0.45)	7.47b (0.72)	8.90a (0.80)	6.31c (0.60)	4.90d (0.44)	4.08e (0.34)
	B	2.89 (0.24)	5.36c (0.25)	6.63b (0.25)	7.38a (0.58)	5.03c (0.22)	4.44c (0.29)	3.46e (0.15)
Ash (g/100g)	A	0.98 (0.05)	1.01bc (0.03)	0.98c (0.03)	0.98c (0.03)	1.04ab (0.02)	1.05ab (0.02)	1.06a (0.02)
	B	1.09 (0.05)	1.08 (0.01)	1.06 (0.01)	1.07 (0.02)	1.08 (0.02)	1.09 (0.02)	1.10 (0.02)
Cholesterol	A	72 (4)	65 (2)	67 (3)	64 (2)	66 (3)	67 (3)	63 (2)
	B	66 (2)	64 (2)	66 (1)	67 (2)	67 (2)	64 (1)	64 (2)
Vit B_6	A	— —	0.15 (0.00)	— —	0.15 (0.01)	— —	— —	— —
	B	— —	0.17 (0.02)	— —	0.19 (0.01)	— —	— —	— —
Vit B_{12} (mcg/100g)	A	2.2 (0.1)	2.3c (0.2)	2.9a (0.3)	2.2bc (0.2)	2.1c (0.1)	2.6ab (0.2)	2.5ab (0.2)
	B	2.7 (0.4)	3.1a (0.2)	2.7ab (0.2)	2.5bc (0.1)	2.3c (0.1)	2.9ab (0.2)	2.7ab (0.2)
Riboflavin	A	0.16 (0.00)	0.19abc (0.01)	0.18bc (0.01)	0.16d (0.01)	0.19bc (0.01)	0.20ab (0.01)	0.21a (0.01)
	B	0.23 (0.01)	0.27b (0.01)	0.25c (0.01)	0.24c (0.01)	0.27b (0.02)	0.30a (0.01)	0.28ab (0.01)
Niacin	A	5.28 (0.72)	5.44a (0.33)	4.47b (0.31)	5.45a (0.40)	6.02a (0.35)	5.51a (0.45)	5.55a (0.21)
	B	5.34 (0.39)	6.56ab (0.34)	5.90b (0.21)	6.32ab (0.39)	7.02a (0.41)	6.84a (0.34)	6.54ab (0.25)
Thiamin	A	0.11 (0.01)	0.13bcd (0.01)	0.12d (0.01)	0.13cd (0.01)	0.14abc (0.01)	0.14a (0.01)	0.14ab (0.01)
	B	0.09 (0.01)	0.11c (0.01)	0.11c (0.01)	0.12c (0.01)	0.13b (0.01)	0.14a (0.01)	0.14ab (0.01)
Folacin (mcg/100g)	A	21.08 (3.36)	24.32 (2.22)	24.39 (2.31)	22.34 (2.08)	23.45 (2.89)	24.41 (2.54)	24.72 (2.52)
	B	22.80 (1.72)	22.94 (1.81)	21.98 (1.07)	20.39 (1.90)	24.15 (1.83)	22.66 (1.10)	21.15 (1.64)
Tocopherol	A	0.21 (0.04)	0.16 (0.43)	0.19 (0.03)	0.18 (0.04)	0.15 (0.03)	0.21 (0.04)	0.17 (0.04)
	B	0.26 (0.02)	0.28 (0.03)	0.24 (0.04)	0.21 (0.03)	0.24 (0.04)	0.27 (0.02)	0.23 (0.03)
Pantothenic acid	A	0.75 (0.02)	0.79a (0.02)	0.74ab (0.02)	0.68b (0.03)	0.68b (0.03)	0.76a (0.02)	0.76a (0.02)
	B	0.67 (0.05)	0.65 (0.03)	0.66 (0.04)	0.62 (0.05)	0.64 (0.04)	0.72 (0.02)	0.64 (0.02)
Na	A	89 (7)	72b (2)	79a (3)	76a (3)	70b (1)	68b (1)	63 (1)
	B	99 (3)	74b (1)	72b (1)	79a (2)	73b (2)	68c (1)	68c (2)

Table 1—Nutrient values of raw separable lean from retail cuts of lamb (continued)

| Nutrient | Age group[f] | Nutrient values (mg/100g lean, except where noted)[abcde] | | | | | | |
		Fore-shank[g]	Arm chop	Blade chop	Rib roast	Loin chop	Leg-sirloin	Leg shank
K	A	324 (10)	335b (8)	309cd (6)	307d (10)	322bc (6)	339a (4)	339a (5)
	B	317 (2)	340ab (4)	326bc (2)	319c (6)	339ab (5)	346ya (6)	350a (6)
Mg	A	23 (0.2)	22b (0.5)	21c (0.4)	22b (0.4)	24a (0.5)	24a (0.6)	24a (0.5)
	B	24 (0.1)	24b (0.4)	23c (0.2)	24b (0.3)	25a (0.3)	25a (0.4)	26a (0.4)
Ca	A	13 (1)	10b (0.4)	17a (1)	12b (1)	10b (0.5)	6c (0.5)	5c (0.4)
	B	16 (1)	17ab (1)	19a (1)	17b (1)	16b (0.6)	8 (0.4)	8c (0.4)
Zn	A	5.2 (0.1)	3.4b (0.1)	4.2a (0.2)	2.8c (0.2)	2.6c (0.2)	3.1b (0.1)	3.2b (0.1)
	B	6.0 (0.2)	4.5b (0.2)	5.5a (0.2)	4.0cd (0.2)	3.3c (0.1)	3.8d (0.1)	4.1c (0.2)
Cu	A	0.14 (0.01)	0.12ab (0.01)	0.11b (0.01)	0.13ab (0.01)	0.14ab (0.01)	0.14a (0.01)	0.12ab (0.01)
	B	0.11 (0.01)	0.11 (0.00)	0.10 (0.01)	0.11 (0.01)	0.12 (0.01)	0.11 (0.01)	0.12 (0.01)
Fe	A	1.4 (0.2)	1.3ab (0.1)	1.2b (0.0)	1.2b (0.0)	1.3ab (0.1)	1.4a (0.0)	1.3ab (0.0)
	B	1.7 (0.1)	1.8ab (0.1)	1.6c (0.0)	1.8c (0.1)	1.8a (0.1)	1.8a (0.1)	1.9a (0.1)
P	A	195 (7)	192b (4)	191b (2)	190b (2)	199a (3)	201a (2)	205a (2)
	B	178 (3)	180b (3)	174bc (5)	172c (5)	180ab (5)	178bc (6)	186a (5)

[abcde] Means within a row followed by different letters differ significantly (P < 0.05).
[f] A = 4–4.5 months; B = 8–9 months.
[g] Foreshank (n = 4) was not part of the statistical analysis due to unequal sampling, n = 8 for all other cuts, except for Vitamin B_6 where n = 4.
[h] Standard error of the mean.

taken immediately after homogenization while those for other analyses were placed in amber vials and kept frozen at −20°C until used.

Analyses

AOAC (1980) methods were used to quantitate moisture (24.002), protein (24.057), ash (24.009), vitamin B_6 (43.188), niacin (43.131), vitamin B_{12} (43.131), folic acid (43.131, after enzymatic hydrolysis), pantothenic acid (43.131), tocopherol (43.106), thiamin (43.029) and riboflavin (43.042). Total lipids were determined by the chloroform:methanol extraction method of Folch et al. (1957). The method of Slover and Lanza (1979) was used to quantitate fatty acids on a 10 meter SP-30 capillary column (Supelco). Cholesterol was quantitated by gas liquid chromatography on a 6-ft packed column (3% SE-30 on 10/20 mesh Gas-Chrom Q) using stigmesterol as an internal standards. Inorganic nutrients, except phosphorus was quantitated by atomic absorption spectrophotometry (Perkin Elmer, 1976) after extraction of dry-ashed residues. Phosphorus was quantitated on the dry-ashed extract by the colorimetric method of Fiske and Subbarow (1925).

Statistical analysis

An analysis of variance was performed using the SAS software system, as described by Goodnight and Sall (1979). Sources of variation considered were age and cuts. Duncan's multiple range test was performed using the appropriate error term. When data were unbalanced, least squares means were completed and the ANOVA and Duncan[s test adjusted accordingly.

RESULTS & DISCUSSION

FIG. 1 SHOWS that the retail cuts under study originated from diverse areas of the carcass so that all sections of the
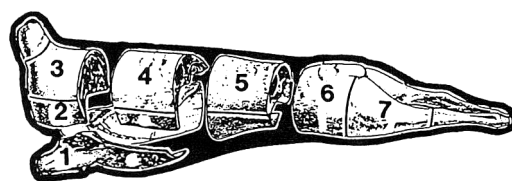
Fig. 1—Anatomical origin of retail cuts of lamb and method of cooking (1) Fore shank (braise); (2) Arm chops (broil); (3) Blade chops (broil); (4) Rib roast (roast); (5) Loin chops (broil); (6) Sirloin half of leg (roast); (7) Shank half of leg (roast).

carcass were well represented. The nutrient values of the raw lean from these cuts, representing the two age groups, are presented in Table 1. It should be noted that although age is a major variable, as judged from some data on beef (Lawrie, 1961; Kotula and Lusby, 1982), there might be other effects such as season which might exert some effects on nutrient composition. None of these effects are separated from the age effect in our presentation. Moisture and total lipids varied inversely to each other with the rib roast containing a significantly (P < 0.05) higher amount of total lipids and a lower amount of moisture compared to the other cuts. There was no cut to cut variation in protein content. Between age groups, the lean from the older lambs contained slightly less total lipids than the younger lambs. Two reasons may account for this. The older lambs could have been stressed during the hot and dry summer of 1981. Growth rate was lower during this period. Mean carcass

Table 2—Nutrient values of cooked lean from retail cuts of lamb

Nutrient	Age group[f]	Fore-shank[g]	Arm chop	Blade chop	Rib roast	Loin chop	Leg-sirloin	Leg-shank
		Nutrient values (mg/100g lean, except where noted)[abcde]						
Moisture (g/100g)	A	62.10 (0.30)[h]	52.40d (0.48)	60.44b (0.48)	57.86c (0.71)	60.06b (0.49)	60.53b (0.53)	63.71a (0.51)
	B	61.48 (0.32)	46.25c (1.91)	64.48a (0.44)	62.39b (0.58)	61.90b (0.40)	64.45a (0.51)	66.13a (0.43)
Protein (g/100g)	A	32.61 (0.91)	35.97a (0.56)	26.71bc (0.74)	25.96c (0.56)	29.77b (0.44)	29.96b (0.54)	28.42b (0.35)
	B	29.42 (3.71)	35.18a (2.01)	25.79c (1.25)	26.36c (0.31)	30.20b (1.23)	27.00bc (0.49)	27.84b (0.53)
Total fat (g/100g)	A	6.47 (0.36)	13.81b (0.57)	12.79b (0.62)	15.16a (0.72)	10.42c (0.73)	10.29c (0.77)	7.00d (0.41)
	B	5.57 (0.28)	14.36a (1.24)	9.65c (0.37)	11.57b (0.50)	8.92cd (0.32)	8.05d (0.46)	6.35e (0.26)
Ash (g/100g)	A	0.91 (0.02)	1.14b (0.02)	1.27a (0.02)	1.05c (0.04)	1.23a (0.04)	1.04c (0.03)	1.07c (0.03)
	B	0.98 (0.04)	1.30a (0.05)	1.24a (0.01)	1.09b (0.01)	1.25a (0.02)	1.12b (0.01)	1.13b (0.01)
Cholesterol	A	106 (8)	119c (3)	96b (3)	92b (2)	97b (4)	100b (3)	95b (2)
	B	102 (9)	124a (6)	86bc (3)	83c (3)	92b (5)	84c (4)	79c (5)
B_6	A	— —	0.13 (0.00)	— —	0.15 (0.01)	— —	— —	— —
	B	— —	0.14 (0.02)	— —	0.16 (0.01)	— —	— —	— —
B_{12} (mcg/100g)	A	2.0 (0.1)	2.5a (0.2)	2.7a (0.2)	1.9b (0.1)	2.2b (0.1)	2.4ab (0.2)	2.6a (0.3)
	B	2.5 (0.1)	2.8 (0.2)	2.9 (0.3)	2.4 (0.2)	2.8 (0.2)	2.7 (0.2)	2.8 (0.2)
Riboflavin	A	0.16 (0.01)	0.21cd (0.02)	0.22bc (0.02)	0.19d (0.01)	0.23abc (0.01)	0.26a (0.01)	0.25a (0.01)
	B	0.23 (0.01)	0.32b (0.02)	0.30b (0.01)	0.28c (0.01)	0.33ab (0.02)	0.36a (0.02)	0.32b (0.01)
Niacin	A	5.1 (0.4)	5.9a (0.3)	5.8a (0.2)	6.1a (0.4)	6.9b (0.4)	6.0a (0.2)	6.3a (0.1)
	B	5.1 (0.7)	6.7 (0.4)	6.3 (0.5)	6.2 (0.4)	6.8 (0.4)	6.5 (0.5)	6.4 (0.3)
Thiamin	A	0.05 (0.01)	0.07c (0.01)	0.10b (0.01)	0.09b (0.01)	0.11a (0.01)	0.11a (0.01)	0.11a (0.01)
	B	0.04 (0.00)	0.06 (0.01)	0.11 (0.01)	0.09 (0.00)	0.12 (0.01)	0.12 (0.00)	0.11 (0.00)
Folacin (mcg/100g)	A	18.40 (1.92)	23.61 (3.30)	22.22 (2.02)	24.40 (2.49)	25.12 (2.00)	22.64 (1.50)	23.95 (1.94)
	B	19.20 (0.92)	21.36 (2.27)	20.00 (1.41)	18.47 (0.97)	23.19 (2.28)	19.82 (1.37)	25.01 (3.04)
Tocopherol	A	0.19 (0.11)	0.18 (0.05)	0.10 (0.02)	0.09 (0.03)	0.07 (0.02)	0.13 (0.03)	0.13 (0.03)
	B	—	0.24 (n = 1)	0.27 (0.04)	0.22 (0.05)	0.26 (0.03)	0.22 (0.05)	0.26 (0.05)
Pantothenic acid	A	0.60 (0.04)	0.64a (0.02)	0.76b (0.04)	0.69ab (0.04)	0.68ab (0.04)	0.75b (0.04)	0.76b (0.02)
	B	0.67 (0.04)	0.60 (0.03)	0.62 (0.03)	0.64 (0.02)	0.64 (0.02)	0.65 (0.04)	0.66 (0.04)
Na	A	74 (2)	72c (1)	94a (4)	79b (2)	84ab (2)	70d (1)	63c (1)
	B	74 (2)	79 (3)	82 (2)	83 (2)	83 (2)	71 (1)	69 (2)

Table 2—Nutrient values of cooked lean from retail cuts of lamb (continued)

Nutrient	Age group[f]	Nutrient values (mg/100g lean, except where noted)[abcde]						
		Fore-shank[g]	Arm chop	Blade chop	Rib roast	Loin chop	Leg-sirloin	Leg-shank
K	A	266 (28)	311c (5)	365a (9)	308c (6)	365a (13)	317bc (3)	329b (5)
	B	269 (34)	365bc (10)	370ab (4)	322d (5)	388a (10)	348c (3)	355c (4)
Mg (mg/100g)	A	22.70 (0.4)	26.50b (0.6)	25.44bc (0.4)	22.41d (0.4)	27.93a (0.5)	24.31c (0.4)	24.98c (0.5)
	B	24 (0.4)	32a (0.6)	27c (0.3)	24d (0.3)	29b (0.8)	26c (0.4)	28c (0.5)
Ca	A	14 (1)	21ab (2)	23a (2)	18bc (2)	15c (0.9)	8d (0.6)	7d (0.3)
	B	25 (1)	30a (0.9)	25b (1)	24b (2)	22b (2)	8c (0.3)	9c (0.3)
Zn	A	7.9 (0.1)	6.2a (0.1)	6.1a (0.2)	3.9c (0.1)	3.9c (0.1)	4.5b (0.2)	4.8b (0.1)
	B	9.4 (0.2)	8.4a (0.3)	6.9b (0.2)	5.0c (0.2)	4.4d (0.1)	5.2c (0.1)	5.3c (0.1)
Cu	A	0.13 (0.01)	0.16a (0.02)	0.13b (0.01)	0.13b (0.01)	0.14ab (0.01)	0.12b (0.01)	0.12b (0.02)
	B	0.12 (0.01)	0.15a (0.01)	0.13ac (0.01)	0.13ac (0.01)	0.15a (0.01)	0.11c (0.01)	0.12bc (0.01)
Fe	A	2.0 (0.1)	2.2a (0.1)	1.6de (0.1)	1.5e (0.0)	1.8cd (0.1)	2.1ab (0.1)	1.9bc (0.1)
	B	2.6 (0.1)	3.2a (0.2)	2.0c (0.1)	2.0c (0.1)	2.2bc (0.1)	2.3b (0.1)	2.2bc (0.1)
P	A	183 (3)	228ab (5)	231a (6)	200d (4)	236a (8)	213c (4)	218bc (2)
	B	167 (6)	236a (10)	201c (4)	189 (6)	215b (7)	194c (5)	198c (6)

abcde Means within a row followed by different letters differ significantly (P < 0.05).
f A = 4–4.5 months; B = 8–9 months.
g The foreshank (n = 4) was not part of the statistical analysis due to unequal samples; n = 8 for all other cuts, except for Vitamin B_6 where n = 4.
h Standard error of the mean.

Table 3—Fatty acid composition of raw separable lean from two age groups of retail cuts of lamb

Retail cuts	Age group[a]	Fatty acids (gm/100 gm lean)												
		10:0	12:0	14:0	14:1	15:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	P/S[b]
Fore shank	A	<.01	<.01	0.11	0.01	0.02	0.65	0.11	0.29	1.39	0.28	0.02	0.06	0.34
	B	<.01	<.01	0.06	<0.01	0.01	0.44	0.07	0.30	1.05	0.16	0.02	0.04	0.27
Arm chop	A	0.01	0.02	0.20	0.02	0.04	1.04	0.16	0.49	2.17	0.38	0.03	0.06	0.29
	B	0.01	0.01	0.13	0.01	0.05	0.98	0.14	0.73	2.18	0.25	0.03	0.03	0.16(0.14)
Blade chop	A	0.02	0.03	0.27	0.02	0.04	1.18	0.21	0.75	3.06	0.41	0.04	0.06	0.22
	B	0.01	0.01	0.16	0.02	0.03	1.24	0.17	1.01	2.77	0.34	0.05	0.05	0.18(0.14)[c]
Rib roast	A	0.02	0.03	0.34	0.02	0.05	1.86	0.26	0.94	3.76	0.56	0.05	0.06	0.21
	B	0.01	0.01	0.19	0.02	0.04	1.48	0.22	1.15	3.18	0.34	0.04	0.04	0.14(0.16)
Loin chop	A	0.01	0.02	0.20	0.02	0.04	1.20	0.16	0.64	2.48	0.41	0.03	0.06	0.24
	B	0.01	0.01	0.11	0.01	0.02	0.95	0.12	0.76	2.07	0.24	0.03	0.04	0.16(0.16)
Leg-sirloin half	A	0.01	0.01	0.15	0.01	0.03	0.86	0.11	0.43	1.77	0.34	0.02	0.06	0.28
	B	0.00	0.01	0.09	0.01	0.02	0.78	0.11	0.61	1.75	0.24	0.03	0.04	0.20(0.16)
Leg-shank half	A	0.01	0.01	0.12	0.01	0.03	0.72	0.10	0.34	1.52	0.29	0.02	0.06	0.30
	B	0.00	0.00	0.07	0.00	0.01	0.59	0.08	0.43	1.34	0.16	0.01	0.04	0.27(0.16)

a A = 4–4.5 month; B = 9 months.
b Ratio of polyunsaturated to saturated fatty acids.
c Values in parenthesis are computed from data of Anderson et al. (1977).

weights for the young lamb was 24.5 kg and 25.0 kg for the old lambs. Also, since Choice grade carcasses were selected for both age groups, the marbling scores would tend to equalize the total lipid values in the lean.

Table 4—Percent true cooking retention of nutrients in the separable lean of retail cuts of lamb

Nutrients	Percent True Retention						
	Fore-shank (Braise)[a]	Arm chop (Braise)	Blade chop (Broil)	Rib roast (Roast)	Loin chop (Broil)	Leg-sirloin (Roast)	Leg-shank (Roast)
Moisture	55 (3)[b]	34 (1)	70 (1)	70 (3)	60 (1)	63 (2)	65 (2)
Protein	99 (6)	91 (3)	98 (2)	108 (4)	105 (3)	101 (3)	106 (3)
Total lipids	130 (13)	136 (10)	115 (5)	146 (12)	126 (5)	156 (11)	137 (7)
Ash	66 (3)	58 (1)	88 (1)	87 (3)	85 (2)	75 (2)	77 (2)
Cholesterol	102 (4)	97 (4)	98 (2)	112 (6)	105 (5)	105 (5)	103 (3)
B_6	— —	45 (2)	— —	75 (6)	— —	— —	— —
B_{12}	64 (4)	51 (2)	74 (4)	79 (7)	84 (6)	70 (3)	79 (6)
Riboflavin	66 (4)	59 (3)	88 (4)	97 (4)	89 (3)	91 (2)	88 (2)
Niacin	66 (6)	55 (4)	86 (5)	88 (6)	78 (5)	76 (5)	78 (2)
Thiamin	29 (3)	28 (3)	61 (4)	64 (4)	63 (4)	61 (4)	61 (5)
Folacin	60 (5)	50 (5)	67 (4)	90 (9)	77 (6)	71 (7)	89 (11)
Tocopherol	— —	55 (7)	67 (14)	54 (11)	64 (11)	54 (7)	68 (10)
Pantothenic acid	61 (4)	44 (2)	71 (3)	89 (6)	73 (3)	69 (2)	78 (5)
Na	54 (7)	76 (2)	88 (3)	81 (2)	84 (1)	71 (1)	66 (1)
K	56 (4)	50 (1)	83 (1)	83 (3)	82 (2)	72 (2)	75 (2)
Mg	68 (3)	63 (1)	85 (1)	85 (3)	84 (2)	76 (1)	80 (2)
Ca	92 (10)	103 (9)	97 (5)	126 (10)	105 (4)	82 (4)	99 (6)
Zn	105 (4)	94 (3)	97 (4)	113 (7)	104 (5)	104 (3)	104 (3)
Cu	73 (6)	68 (4)	89 (6)	96 (7)	87 (6)	70 (4)	77 (7)
Fe	98 (6)	90 (5)	92 (2)	103 (5)	94 (4)	105 (5)	100 (4)
P	63 (2)	63 (1)	85 (1)	89 (3)	86 (2)	79 (1)	81 (2)

[a] n = 8 for fore shank; n = 16 for all other cuts, except Vitamin B_6 where n = 8.
[b] Numbers in parenthesis are the standard errors of the means.

Excluding the fore shank (Table 1), which was not part of the statistical analysis due to unequal sampling, there were no significant differences (P > 0.05) in cholesterol values for uncooked lean between cuts or age groups. The values agree closely with the 70 mg/100g reported by Feeley et al., 1972.

There were several statistically significant differences in the vitamin contents between cuts (Table 1) but these differences were very small and would not have any practical significance. Although there is no definition of what is practically significant, a difference of less than 5% of the Recommended Dietary Allowance (NAS-NRC, 1980) is considered to be of little practical nutritional significance

for our discussion. This criterion is in line with guidelines set up by Stewart (1979). In the discussions to follow, the RDA of males, age 23–50 (NAS-NRC, 1980), will be used as a reference point for comparison and a value of 3.5 oz (100g) will be used as an average serving. The uncooked lean of the older group had significantly (P < 0.05) higher amounts of riboflavin, niacin and tocopherol. Differences in riboflavin between age groups for all cuts range from 4.4 to 6.2 percent of the RDA.

The values for inorganic nutrients in uncooked lean (Table 1) show statistical differences (P < 0.05) between cuts, but except for Zn, the differences were very small in relation to the RDA for these nutrients. The blade chops

Age group[a]	Kcal/100g lean						
	Fore shank (Braise)[b]	Arm chop (Braise)	Blade chop (Broil)	Rib roast (Roast)	Loin chop (Broil)	Leg-sirloin (Roast)	Leg-shank (Roast)
A	198	277	229	248	221	221	184
B	176	280	197	217	211	187	176

[a] A = 4–4.5 months; B = 8–9 months.
[b] n = 4 for fore shank and n = 8 for all other cuts.

showed the highest values for Zn and the loin chops the lowest. The differences between the two values were 10.9 for the young lambs and 15.1 percent of the RDA for the older lambs, respectively. In all cases, cuts from the older lambs exhibited higher levels of Zn than those from younger lambs. Zinc values from young lambs agree closely with the value of 3.0 mg/100g reported by Murphy et al. (1975b). Increase in Zn levels with increasing age has been reported for bovine muscle by Kotula and Lusby (1982). Iron levels varied between cuts but the differences were small. The largest difference, amounting to only 2.4% of the RDA was found between the leg-shank and blade chops from the older lambs. The older lambs had higher iron values for all cuts but, again, the differences between age groups which ranged from 4.3–5.7% of the RDA were small. The increase in iron content in bovine muscle with increasing age has been well documented by Lawrie (1961).

The nutrient values of cooked lamb are more useable by consumers than raw values. While the raw values provide base-line information to evaluate production and marketing effects on nutrient composition, the cooked values provide information on what is consumed. The nutrient compositions of cooked lean from retail cuts of lamb are presented in Table 2. The differences in nutrient values between cuts and between age groups follow the same trend as seen with the raw cuts. However, the magnitude of the differences between cuts differ due to the variations in nutrient retention with different cooking methods. The components exhibiting the greatest differences between cuts were moisture, protein, total lipids, Zn and Fe. For the young lambs moisture levels in separable lean in the arm chop were 81.7% of the shank half of leg and for the older lambs, it was 70.0 percent. Also, in the young lambs total lipids in the shank half of leg were 46.2% of the rib roast and for the older lambs it was 44.2% of the arm chops. Differences can not be expressed as a percent of the RDA because no RDA's are available for moisture and lipids. Protein levels between arm chop and rib roast showed the greatest difference in both the young and older lambs. Differences in protein levels were 17.9 and 15.8% of the RDA for the young and old lambs, respectively. For the inorganic nutrients, the variations in zinc and iron levels between cuts are the most noteworthy. Differences in zinc levels were 15.6 to 26.7 percent of the RDA between the arm and loin chops for the young and older lambs, respectively. For iron, differences were 6.7–12.1% of the RDA between the arm chop and rib roast for the young and older lambs, respectively.

Fatty acid values for raw retail cuts of lamb are presented in Table 3. Most of the values, where comparisons are possible, agree well with those reported by Anderson et al. (1977). One of the notable exceptions is in the levels of linoleic (18:2) acid. Cuts from the younger lambs had higher values of this acid than the older lambs. Also, values from the younger lambs were higher than those reported by Anderson et al. (1977). The difference is reflected in the higher P/S ratio for the younger lambs than for the others. Myristic acid (C14:0) was higher in cuts from younger than older lambs. Cramer and Marchello (1964) reported similar findings with subcutaneous fat. They found that

myristic acid in biopsied subcutaneous fat from pre-weaned lambs was approximately 14% of the total fatty acids but after 18 months, the level had dropped to about 5%.

The amount of nutrients retained after cooking is best described by true retention factors (Murphy et al., 1975a). The true retention factors presented in Table 4 must be considered to be guidelines and not absolute since variations in cooking methods can affect the retention of certain nutrients (Cover and Dilsaver, 1947; Engler and Bowers, 1976; Moss et al., 1983). In Table 4, values for the two age groups were combined since in most cases the values were close and no age effect was evident. Generally, the vitamins are the least retained with the largest loss occurring for thiamin. In most cases, cuts cooked by braising retained the least amounts of nutrients. Moss and coworkers (1983) found similar trends with retail pork. Retentions for total fat exceeded 100%. Again, similar results have been reported by Moss et al. (1983) for pork. The increase is believed to be due to the absorption of the separable fat into the lean during cooking. The total fat retention for the roasted samples was higher than for the braised and broiled samples. During roasting, the samples were placed in the oven with the subcutaneous fat side up which resulted in fat migration into the lean from the subcutaneous fat and intermuscular fat while for braising and broiling, the subcutaneous fat was to the side so the principal contribution to the additional fat in the lean came from the intermuscular fat. Consequently, broiling and braising result in less fat retention than cooking by roasting.

The caloric values of the cooked lean from the retail cuts are presented in Table 5. Calculations of energy values were based on the Atwater system as described by Merrill and Watt (1973). The lean from the older animals tended to have less calories than from the younger animals but the differences were very small. For the blade chops where the values varied the most between age groups, the difference was only 1.5% of a daily energy intake of 2200 kcal. Between cuts, the maximum difference was 103.8 kcal which occurred between the fore shank and arm chop in the older group of lambs. This difference, based on 100g of cooked lean, is 4.7% of the energy intake of 220 kcal. Again, the difference is of little practical significance.

In summary, except for moisture, total lipids, riboflavin, niacin, Zn and Fe, there were no practical differences in nutrients between cuts or between age groups. Thiamin was the most labile nutrient during cooking. The young lambs had higher linoleic acid content in the lean than previously reported. Based on the cooking conditions described in this experiment, a daily serving of 3 oz could result in calorie intake of 149.5–237.8 kcal, depending on which of the seven cuts is consumed. Wherever comparisons were possible, our results agreed well with those reported in Handbook 8 (Watt and Merrill, 1967).

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Modification of Egg White Proteins with Oleic Acid

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———————————— ABSTRACT ————————————

Studies on the chemical modification of egg white with oleic acid (5–50 moles/50,000g of egg white protein) revealed that the reagent partitioned equally between supernatant and precipitate. The mole ratio of oleic acid to protein in solution at the 20 mole level of treatment was 14.6:1. Oleic acid did not selectively precipitate ovalbumin, conalbumin, or lysozyme. An increase in negative charge of proteins was observed in the chromatograms of treated egg white. No difference in molecular weights of treated egg white proteins was observed. The viscosity varied with the concentration of the reagent. In cooked, frozen, and thawed egg white water retaining index was 1.5–10X greater for treated than untreated material.

INTRODUCTION

MANY INVESTIGATIONS have been conducted to examine the altered functional properties of proteins and food systems treated with various acylating reagents (Gandhi et al., 1968; Grant, 1973; Melnychyn and Stapley, 1973; Chen et al., 1975; Ball and Winn, 1982). Several researchers have suggested that detergents or fatty acids could also be useful modifiers. Lundgren and O'Connell (1944) treated ovalbumin and keratin of chicken feathers with a detergent, alkyl benzenesulfonate, to produce artificial fibers with the strength and moisture-absorbing qualities similar to those of silk and wool. They suggested that proteins derived from inedible technical grade egg white and other industrial commodities could be converted to useful fibers. In a more recent study, the true shear strain of heated egg white gels was higher for oleic acid treatment than control (Montejano-Gaitan et al., 1982). This measurement suggested that the biophysical properties of the proteins had been sufficiently altered so that gels containing treated materials were more elastic and would deform to a greater extent before breakage.

Boyer et al. (1946a, b) observed that low concentrations of fatty acids could stabilize bovine serum albumin against denaturation by heat, urea, or guanidine. Hegg and Lofquist (1974) reported that the aggregation temperature of crude ovalbumin was elevated in the presence of low concentrations of sodium dodecyl sulfate, 2-decylcitric acid, and lauric acid. These investigators proposed using fatty acids to obtain comparable results.

It has been recommended that 0.02–0.03% oleic acid with small amounts of glycerol be added to egg white to improve cake texture (Gardner, 1960). Several early patents also advocated the use of polyhydric alcohols in improving the texture of cakes (Katzman, 1939; Shaffer, 1956).

The overall implication from these studies is that treatment of proteins or food systems with detergents or fatty acids may have varied industrial applications. Moreover, investigating the attributes of chemical modification with these reagents could (a) provide basic knowledge on protein structure as it relates to function, and (b) increase the understanding of lipid-protein interactions. The objectives of the study were to determine properties of egg white proteins modified by the addition of oleic acid to egg white. For purposes of comparison, less extensive studies of the reaction between egg white and two other reagents, sodium dodecyl sulfate (SDS) and methyl oleate, were performed.

MATERIALS & METHODS

Preparation of treated egg white

Egg white was separated from the yolk of 1 day old eggs (Poultry Science Dept., NCSU, Raleigh, NC 27695), blended, frozen, and held at −20°C until needed. Samples were thawed at 20–24°C. Oleic acid and methyl oleate (Fisher Scientific Co., Pittsburg, PA; Nu Chek-Prep., Inc., Elysian, MN) 1-^{14}C oleic acid (New England Nuclear Corp., Boston, MA) and SDS (Bio-Rad, Richmond, CA) were added in increments with continuous stirring to obtain 5–50 moles of reagent/50,000g egg white protein. Initial pH of egg white was 8.7–9. Reaction temperature was maintained at 12 ± 2°C, and pH was not allowed to drop below 7.5. Intermittent and final pH was adjusted with 1N NaOH or 1N HCl. After 24 hr storage at 10°C, pH was adjusted to 8.5 and the material was centrifuged at 12,000 × g for 30 min to remove precipitated material. Supernatant and precipitate were stored at −20°C until further analyses could be performed.

Quantitative analyses

Egg white was weighed into tared centrifuge tubes. After centrifugation the supernatant was separated from precipitate. Precipitate was weighed and supernatant was determined by difference. Protein was determined by micro-Kjeldahl method (AOAC, 1980). Partitioning of 1-^{14}C oleic acid between supernatant and precipitate was estimated by combusting samples in triplicate in a biological material oxidizer (Model OXO1, R.J. Harvey Instruments, Hillsdale, NY). Oxiflour-CO_2 (New England Nuclear Corp., Boston, MA) was the collecting scintillant. A liquid scintillation spectrophotometer (Model 574, Packard Instrument Co., Inc., Downers Grove, IL) was used to measure radioactivity.

Separation of egg white proteins

DEAE-Sephacel chromatography was performed by the method of Palladino et al. (1981). DEAE-Sephacel (Pharmacia, Uppsala, Sweden) was washed with 0.5M glycine to attain a pH of 5.8. A 2.5 × 40 cm column was packed with the ion exchanger at 1.8 mL/min. One mL of egg white diluted 1:1 with pH 5.8 glycine buffer was separated into various fractions by continuous gradient elution at 1.5 mL/min. Fraction volume was 8 mL each. The gradient was formed with glycine buffers of pH 5.8, 6.8, 4.4 and 2.5, respectively.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out on 80 × 5.5 mm tube gels (12.5%) in an electrophoresis cell (Model 150A, Bio-Rad, Richmond, CA). Gel stock solutions and buffers were made according to the method of Porzio and Pearson (1977). Samples of whole egg white proteins (20 mg protein/mL) and protein standards (0.05 μg protein/mL) were dissolved in a solution of 2.5% SDS, 0.1M tris, pH 9.0 (Fisher Scientific Co., Pittsburgh, PA), 20 mM ethylenediamine-tetraacetic acid (Fisher Scientific Co., Pittsburgh, PA), and 20 mM dithiothreitol (Bio-Rad, Richmond, CA) and mixed 1:1 with 0.01% bromophenol blue (Fisher Scientific Co., Pittsburgh, PA) before applying to the gels. After a 6–8 hr run at 1 mA/gel, the dye front was marked with a thin copper wire. Protein bands were stained and destained by the method of Weber et al. (1972). Unstained and stained gels were scanned (Guilford Microprocessor-Controlled Spectrophotometer System 2600, Oberlin, OH) at 280 and 600 nm respectively and absorbance was recorded (Hewlett Packard Plotter, Model 7225B, Oberlin, OH). R_f values were determined from the plots relative to

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the dye front. Molecular weights of modified egg white proteins were estimated by comparing their R_f values to R_f values of lysozyme, soybean trypsin inhibitor, carbonic anhydrase, ovalbumin, bovine serum albumin, and phosphorylase B prepared for use as molecular weight standards (Bio-Rad, Richmond, CA).

Water-retaining index (WRI)

Twenty mL of egg white in aluminum weighing pans (50 mm diameter) were covered and cooked over steam for 7 min to an end point temperature of 90°C. After cooling to room temperature, a cylinder 1 cm diameter x 1 cm long of cooked material was placed on 11 cm diameter No. 1 Whatman filter paper and pressed between plexiglass plates in a Carver press (Model 22200-220, Fred S. Carver, Inc., Summit, NJ) at 8.7×10^6 Pa for 1 min. Area of the pressed egg white and released water were measured with a polar compensating planimeter (Model 39231, Gelman Instrument Co., Ann Harbor, MI). WRI was determined by a modification of the procedures of Wismer-Pedersen (1971) and Hoffman et al. (1982). WRI was defined as A_{ew}/A_{pw} where A_{ew} was the area of the pressed egg white and A_{pw} was the total area of A_{ew} and the surrounding water film. WRI was a parameter used to measure the freeze-thaw stability of egg white gels. Samples were frozen at -20°C in still air and thawed at room temperature.

Absolute viscosity

Cannon-Fenske viscometers were used to measure viscosity. Constants for each viscometer were approximated from available data (Cannon and Manning, 1954a, b; and personal correspondence). Absolute viscosity was calculated as: centipose = efflux time (sec) x constant x density (CRC Handbook of Chemistry and Physics, 1967).

Statistical analyses

Analysis of variance (Snedecor and Cochran, 1973) and Waller-Duncan K-ratio T test (SAS Institute Inc. User's Guide: Statistics, 1982) were used to analyze data.

RESULTS & DISCUSSION

OLEIC ACID AND SDS were added to egg white at concentrations above the critical micelle concentrations as determined by Tortoro et al. (1978) and Fukushima et al. (1981). As the reagents were added, egg white became opaque and a precipitate formed. The amount of precipitate produced at various concentrations of each reagent was determined. A linear relationship existed between the amount of precipitate produced and the level of oleic acid added (Fig. 1). Note that SDS at 15 moles produced precipitate (17.9g)

equal to that of 25 moles oleic acid (17.9g). Presumably, SDS was more reactive than oleic acid. Several investigators have found that sulfates disrupt the structure of serum bovine albumin more than do other hydrocarbons such as alcohols and carboxylates (Ray et al., 1966; Reynolds et al., 1967). We have assumed that the increase in the amount of precipitate formed was the result of the denaturation of proteins by the reagents employed. However, it is possible that centrifugation may have caused greater aggregation of complexes of oleic acid and protein. A linear relationship between the amount of reagent added and the size of the aggregate and quantity of protein therein could have existed.

Partitioning of egg white protein between precipitate and supernatant after treatment with various reagents is shown in Table 1. The values are based on the weight distribution of egg white between precipitate and supernatant and the protein content of the components. Most of the protein remained soluble at each level of treatment. At 20 moles of oleic acid, 80.6% of the protein remained in solution. At the highest concentration of oleic acid (50 moles/50,000g protein), 75.3% of the protein was soluble. SDS at 10 moles precipitated protein (23.7%) similar to that of oleic acid at 50 moles (24.7%). Methyl oleate (25 moles) precipitates 10.8% protein, about equal to that of 5 moles of oleic acid.

Table 1—Partitioning of total egg protein in precipitate and supernatant after treatment with various concentrations of oleic acid, methyl oleate, and sodium dodecyl sulfate

	Moles of reagent per 50,000g egg white protein	Percent of total egg white protein[a,b]	
		In precipitate	In supernatant
Oleic acid	5	11.8	88.2
	15	20.4	79.6
	20	19.4	80.6
	25	19.4	80.6
	50	24.7	75.3
Methyl oleate	25	10.8	89.2
Sodium dodecyl sulfate	5	16.1	83.9
	10	23.7	76.3

[a] 9.3g total protein in 100g egg white.
[b] Average of 6 determinations.

Fig. 1—Percent precipitate from egg white treated with various concentrations of oleic acid, •; sodium dodecyl sulfate, o; and methyl oleate, □. Regression equation for the line is y = 0.32X + 9.5.
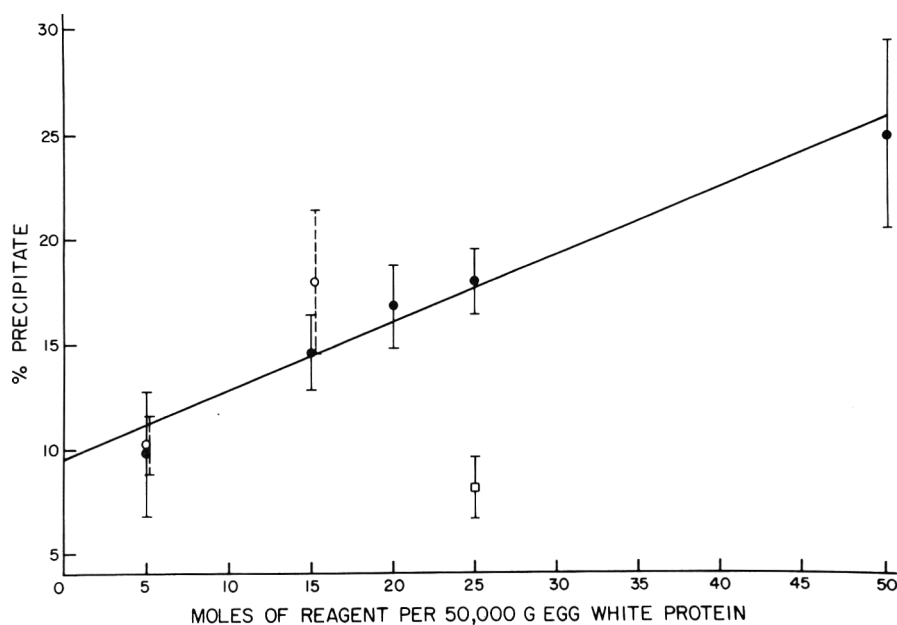
To assess whether oleic acid selectively precipitated any of the major egg white proteins, SDS-PAGE was carried out on samples of protein standards, untreated albumen, and supernatant and precipitate obtained from treatment with 20 moles oleic acid (Fig. 2). Ovalbumin, conalbumin, and lysozyme were identified by comparison to standard proteins. Evaluation of the gel scans of supernatant and precipitate from treated egg white revealed that these three proteins were also present in both fractions. Though a portion of each protein evaluated was precipitable, oleic acid did not selectively precipitate ovalbumin, conalbumin, or lysozyme. The SDS-PAGE data also suggests that within the limits of the methodology, that modification did not alter molecular weights of ovalbumin, conalbumin or lysozyme.

Studies using $1-{}^{14}C$ oleic acid, at the 12.5 and 20 mole concentration, demonstrated that oleic acid partitioned about equally between supernatant and precipitate (Table 2). Molar ratios of oleic acid to protein in each part at the two concentrations were estimated (Table 2). Approximately 3.5 and 5.6X more oleic acid was in the precipitate than in the supernatant for 20 and 12.5 moles respectively. This finding indicated that as the concentration of oleic acid was increased, more of it was associated with the proteins in the supernatant of treated egg white.

Samples of control and treated egg white were chromatographed on DEAE-Sephacel. The elution profile of proteins (Fig. 3) in treated egg white was apparently influenced by several different factors. Oleic acid (pH 3.2) as free micelles probably affected the pH of the eluting buffers and thus the shape of the chromatogram. The first 80 mL of buffer eluted from the column had a pH ranging from 4.0-5.0 instead of pH 5.8-6.8 as expected. The shape of the chromatogram was also influenced by oleic acid-protein complexes. Several investigators have suggested that the detergent protein complex is a type of micelle with protein at its core (Fukushima et al., 1981; Pitts-Rivers and Impiombata, 1968; Fish et al., 1968). Pitts-Rivers and Impiombata have envisioned beads of SDS micelles packed along the polypeptide chain.

The results from SDS-PAGE of the chromatographic fractions (Fig. 3), indicated that two types of oleic acid-protein complexes could have been formed. The first type of complex could have been composed of two or more different proteins associating with each other and surrounded by oleic acid. SDS-PAGE revealed that lysozyme was found in every fraction, excluding fraction A. Lysozyme and ovalbumin were present in fractions E, H, and I while lysozyme and conalbumin were present in fraction G. Vadehra and Nath (1973) have reported that lysozyme due to its basic nature has been shown to bind conalbumin, ovalbumin, and ovomucin. It is possible that initial complexes of lysozyme and ovalbumin or lysozyme and conalbumin were surrounded by oleic acid and were eluted on DEAE-Sephacel as one complex without being separated into component proteins in the usual manner as indicated by arrows in Fig. 3. A second type of complex could have been composed of one or more molecules of the same protein and oleic acid. This type of complex was reported by Hughey and Curthoys (1976) in their study of δ-glutamyltranspeptidase and Triton X-100. In our study, the amount of oleic acid bound to individual molecules of the same protein would undoubtedly have affected the time of elution. Furthermore, it is not likely that the full extent of the effect of oleic acid was reflected since during chromatography dilution by buffers or acidic pH of buffers could have caused partial and variable reversal of the treatment effect. Houghten and Chramback (1979) have noted that the effect of oleic acid was reversed under acidic conditions, while other workers (Bull and Breese, 1967; Lewis et al., 1967) have reported that oleic acid can be disassociated from prolactin by dialysis.

The complexes of protein and oleic acid were apparently negatively charged. The most striking example of an increase in charge was that lysozyme, usually eluted after the void volume of buffer, was present in almost all of the fractions (Fig. 3). Conalbumin and ovalbumin of treated egg white were also eluted at more negative pH values. Other researchers using DEAE-Sephacel and DEAE-cellulose along with PAGE and SDS-PAGE, respectively, have shown that untreated albumen does not contain proteins having the degree of variation in charge observed (Fig. 3) for treated egg white proteins (McKinney, 1977; Palladino et al., 1981).

To determine if oleic acid was preventing adequate binding of SDS in the SDS-PAGE procedure and thereby interfering with the mobility of the protein identified as
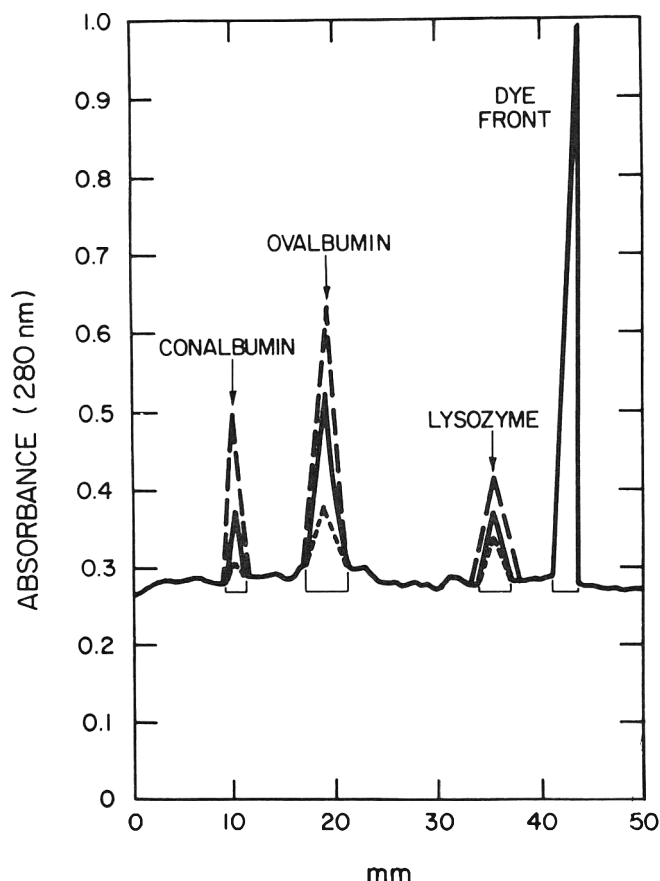
Fig. 2—UV scan of gels containing whole egg white and the supernatant and precipitate from egg white treated with 20 moles of oleic acid. SDS-PAGE was performed on all samples: whole egg white, ——; supernatant, - - -; and precipitate, ···.

Table 2—Partitioning of $1-{}^{14}C$ oleic acid between supernatant and precipitate from egg white treated with 12.5 and 20 moles of oleic acid

Moles of oleic acid per 50,000g egg white protein	Section of treated egg white	% Distribution of oleic acid[a]	Moles oleic acid: 50,000g egg white protein
12.5	Supernatant	47	7.6:1
	Precipitate	53	42.7:1
20.0	Supernatant	54	14.6:1
	Precipitate	46	51.0:1

[a] Average of 10 determinations.

SUPERNATANT FROM EGG WHITE
TREATED WITH OLEIC ACID

A - UNIDENTIFIED
    OVOMUCOID
B - GLOBULINS
    LYSOZYME
C - AVIDIN
    LYSOZYME
D - GLOBULINS
E - OVALBUMIN
    LYSOZYME
F - LYSOZYME
    AVIDIN
G - CONALBUMIN
    LYSOZYME
    AVIDIN
H - OVALBUMIN
    LYSOZYME
I - FLAVOPROTEIN
    OVALBUMIN
    LYSOZYME
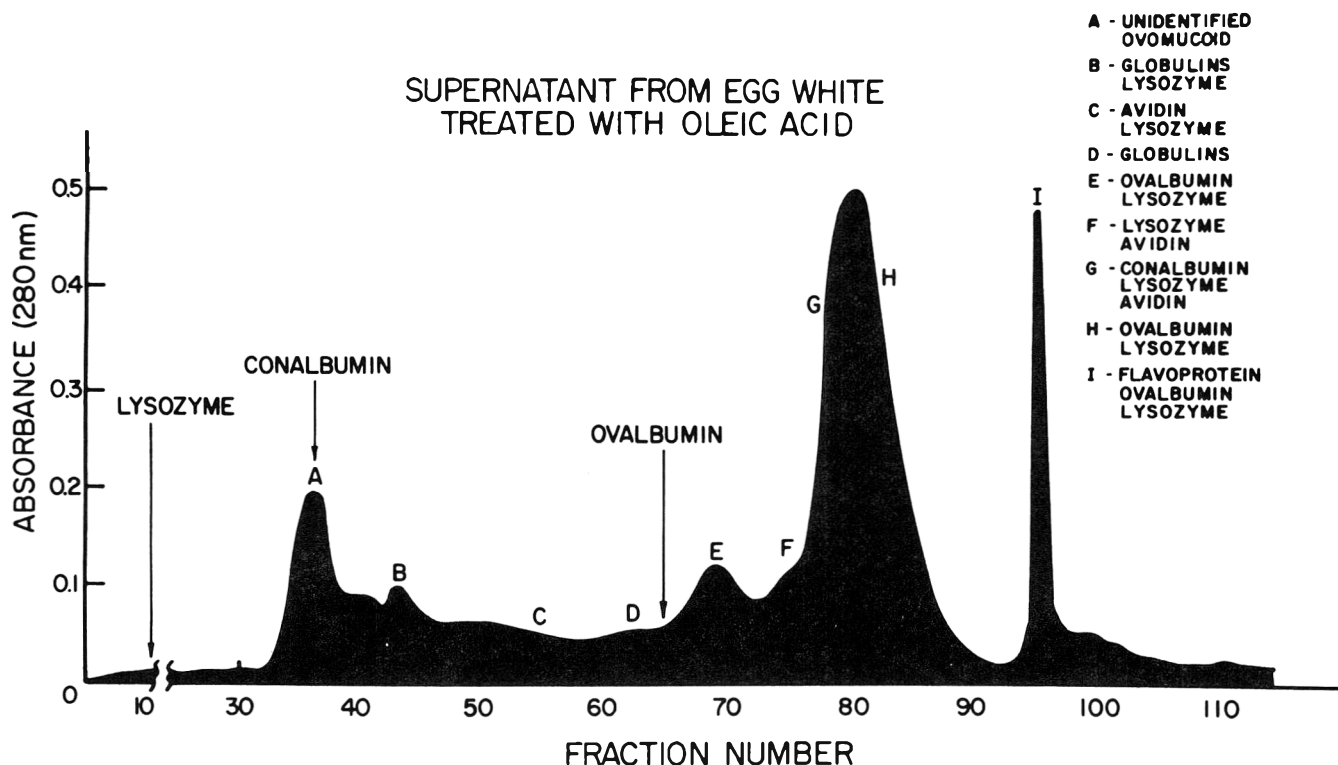
Fig. 3—DEAE cellulose chromatograms of supernatant obtained from the 20 mole level of oleic acid. SDS-PAGE was performed to identify proteins in fractions A-I. Arrows indicate the usual elution sequence for lysozyme, conalbumin, and ovalbumin in untreated egg white.

lysozyme from chromatographic fractions, samples of commercially purified lysozyme were modified with oleic acid and run against untreated samples on SDS-PAGE. There were no differences in mobilities of treated and untreated proteins, indicating that oleic acid was not interfering with SDS binding. In our study on the precipitation of proteins by oleic acid (see Fig. 2) it was also found that there was no difference in binding of SDS to proteins (electrophoretic mobility) in the treated whole egg white compared to untreated samples. Our results indicated that while molecular weights of proteins were not changed, differently charged species were formed after modification with oleic acid.

The absolute viscosity of egg white treated with oleic acid (5-25 moles/50,000g protein) and SDS (5 moles/ 50,000g protein) was lower than that of untreated material (Fig. 4). The viscosity of egg white treated with 25 moles methyl oleate was intermediate between that of 15 and 25 moles oleic acid. At the 15 mole level of SDS, gelation occurred before treatment was completed, therefore it was impossible to determine viscosity. As shown in Fig. 4, egg white treated with 50 moles of oleic acid showed a steady increase in viscosity after 24 hr and eventually gelled on the 6th or 7th day after treatment.

Viscosity changes for 5-25 moles of oleic acid and 5 moles of SDS were probably manifestations of the effect of oleic acid micelles and micellular complexes of oleic acid and protein. Assuming sphericity of micelles and complexes, the intrinsic viscosity would have been approximately 2.5 mL/g (Van Holde, 1971). The reported values for intrinsic viscosity of egg white proteins are higher than that of spherical micelles (Vadehra and Nath, 1973; Nakamura and Ishimaru, 1981). Therefore, the viscosity of treated egg white, as has been demonstrated in our study, would be higher than that of albumen treated at low concentrations of reagents. At higher concentrations of oleic acid more reagent remained in solution as shown in Table 2. Thus aggregation of micelles alone or with oleic acid-pro-
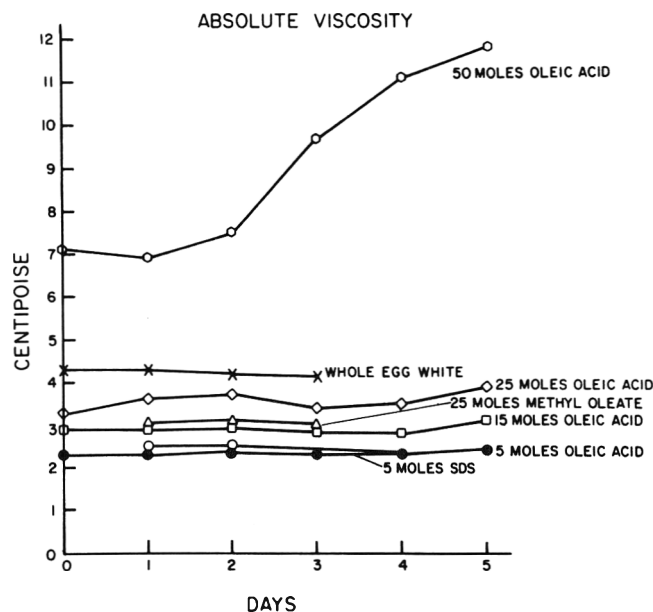
Fig. 4—Absolute viscosity of egg white treated with various levels of oleic acid, methyl oleate, and sodium dodecyl sulfate. Data for 20 moles of oleic acid were the same as that for 15 moles of oleic acid.

tein complexes remaining in solution could have produced the increase in viscosity observed. Since SDS was more reactive than oleic acid, it caused aggregation and subsequent elevation in viscosity at a lower concentration of 15 moles/50,000g egg white protein.

Cooked, frozen egg white has poor freeze-thaw characteristics: it is rubbery and water weeps from the crevices created by ice crystal formation (Davis et al., 1952). Hawley (1970) impeded syneresis in this type product by adding 2-4% carbohydrate which bound water and pre-

Table 3—Water-retaining index (WRI) of supernatant from egg white treated with various levels of oleic acid, methyl oleate, and sodium dodecyl sulfate

Reagent	Moles of reagent per 50,000g egg white protein	WRI[h]	
		Cooked, unfrozen	Cooked, frozen/thawed
Control		0.29[f] ± 0.06	0.10[g] ± 0.03
Oleic acid	5	0.45[e] ± 0.12	0.15[g] ± 0.04
	15	0.83[c] ± 0.17	0.28[f] ± 0.08
	20	0.92[b] ± 0.08	0.84[c] ± 0.17
	25	1.00[a] ± 0.01	1.00[a] ± 0.01
	50	1.00[a] ± 0.01	1.00[a] ± 0.01
Methyl oleate	25	0.33[f] ± 0.06	0.14[g] ± 0.03
Sodium dodecyl sulfate	5	0.42[e] ± 0.09	0.30[f] ± 0.08
	15	0.69[d] ± 0.10	0.73[d] ± 0.19

[a-g] Means with the same letters are not significantly different, α 0.01.
[h] Mean of 10 determinations.

vented layer formation during freezing and consequent syneresis upon thawing. Oleic acid and SDS had a similar effect on the freeze-thaw stability of cooked egg white as indicated by the WRI's (Table 3). Treated, cooked, and frozen albumen exhibited no visual evidence of structural damage after thawing. SDS had a greater effect at 5 and 10 moles than did oleic acid at the same concentration (Table 3). At 25 and 50 moles of oleic acid the WRI for cooked, frozen, and thawed material was 10X greater than that of the control.

Oleic acid and SDS also increased the WRI of cooked unfrozen egg white (Table 3). At the 5 mole concentration both reagents had a WRI 1.6X greater than that of the control. However, 15 moles oleic acid increased the WRI (0.83 ± 0.17) more than did 10 moles of SDS (0.69 ± 0.10). For 20 moles of oleic acid/50,000g protein, the WRI was 1.00 ± 0.01 for cooked unfrozen egg white. Methyl oleate did not significantly increase the WRI of either type of cooked egg white.

The results presented above confirm that oleic acid alters biophysical characteristics of proteins. Modification increased the negative charge on proteins and greatly improved the freeze-thaw characteristics of cooked and frozen albumen. This suggested that an increase in negative charge alone or in combination with expansion of molecular size as obtained by succinylation (Palladino, 1980) is related to greater water binding capacity. The viscosity of treated material was also altered. Further studies on egg white proteins modified with oleic acid will aid in understanding the changes in functional properties observed.

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Cholesterol Oxides in Commercial Dry Egg Products: Isolation and Identification

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———————ABSTRACT———————

5α-Cholestan-5,6α-epoxy-3β-ol (cholesterol α-oxide) and 5β-chole-stan-5,6β-epoxy-3β-ol (cholesterol β-oxide) were isolated from freshly dehydrated commercial whole egg and yolk powders. The isolates were shown to have identical HPLC retention volumes to those of synthesized cholesterol α- and β-oxides, respectively. Their mass (MS), nuclear magnetic resonance (NMR) and infrared (IR) spectra were also proven to be identical. Cholesterol oxides found in dehydrated egg products may have been formed during the commercial drying process since no oxides were found in fresh shell eggs nor in the egg samples lyophilized in the laboratory.

INTRODUCTION

AMONG THE FIFTY or more reported cholesterol oxidation products (Bergstrom and Samuelsson, 1961; Horvath, 1966; Fioriti and Sims, 1967; Chicoye et al., 1968a, b; van Lier and Smith, 1970; Smith and Hill, 1972; and Smith, 1981), cholesterol α-oxide is one of the few with reported physiological activities. Cholesterol α-oxide was reported to induce tumor formation in rats and mice after subcutaneous administration in both oil and aqueous vehicles (Bischoff, 1969). It was found in ultraviolet irradiated human skin (Black and Lo, 1971) and in the skin of hairless mice (Black and Douglas, 1972) and thus was suspected to play a role in skin carcinogenesis (Black and Chen, 1976). Cholesterol α-oxide was found in the sera of hypercholesterolemia patients but not in healthy persons (Gray and Lawrie, 1971) and was suggested to be involved in the initiation of atherosclerotic lesions (Benditt, 1977). The reports on the mutagenicity tests of cholesterol α-oxide were inconsistent. Smith et al. (1979), Kadis (1978), and Kelsey and Pienta (1979) reported negative response of *S. typhimurium* to cholesterol α-oxide, but Blackburn et al. (1979) reported an opposite finding. Other in vitro studies, such as the association of cholesterol α-oxide with DNA (Blackburn et al., 1979), its initiation of DNA repair synthesis in cultured human fibroblasts (Parson and Goss, 1978) and its inhibition of 3-hydroxy-3-methylglutaryl Coenzyme A reductase in cultured mammalian cells (Kandutch and Chen, 1978) are indicative of potential deleterious effect of cholesterol α-oxide to human health.

Despite its potential physiological effects, the presence or absence of cholesterol α-oxide in foods, specifically those rich in cholesterol content, has not been examined. Egg yolk is one of the traditional foods which contains high concentration of cholesterol (Watt and Merrill, 1963; USDA, 1976). Dehydrated yolk products are popular ingredients in many daily dietary items such as bakery products, salad dressings, noodles, baby foods, cake mixes, military rations, etc.

Commercial dehydrated whole egg and egg yolk are produced by a spray-drying process (Bergquist, 1973). During drying, egg yolk is atomized and heated by hot air. The enormous interface area created by the atomization and the high operating temperature (about 60°C) accelerate

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reactions between lipids and molecular oxygen. Chicoye et al. (1968a) reported finding cholesterol β-oxide in spray-dried egg yolk that had been exposed to sun or fluorescent lights, but no cholesterol α-oxide was found in any of their samples. However, lack of apparent cholesterol α-oxide may have been due to difficulty in separating α- and β-isomers. Smith and Kulig (1975) reported that α- and β-oxides were found in a ratio of 1 to 8-11 in the autoxidation mixture of cholesterol.

After a direct separation of cholesterol α-oxide from its β-isomer was successfully achieved (Tsai and Hudson, 1981), a study of the content of cholesterol oxides in commercial yolk products was initiated. This article reports the isolation and identification of cholesterol oxides in freshly produced commercial egg products. Subsequently a study of the distribution of cholesterol oxides in commercial egg products will be reported.

MATERIALS & METHODS

Materials

Spray-dried whole egg and yolk products were sampled off the production line, sealed in plastic bags and shipped to the USDA Western Regional Research Center (Albany, CA) by parcel post. A total of 39 samples from 15 dehydration plants in the United States was received, along with information regarding additives, equipment used, processing conditions, and packaging. The samples were stored between 1-6 months at −21°C in the dark until analyzed. Scrambled Egg Mixes (USDA, 1973) were obtained from storage through the courtesy of the Poultry Division of Agricultural Marketing Service, USDA. The Scrambled Egg Mixes were packed in nitrogen filled aluminum foil laminated pouches, and had been held in warehouses for 3-5 yr.

Cholesterol α-oxide (M.P. 137-139°C) was purchased from Steraloids Inc. (Wilton, NH). The product was found by HPLC analysis to contain 5% β-oxide and was free from other contaminants (Tsai et al., 1980). Radioactive tritium-labeled cholesterol α-oxide was synthesized from [1α, 2α (n) − ³H] cholesterol (Amersham Corp. Arlington Heights, IL), by the method of Fieser and Fieser (1967). The specificity of the synthesized radioactive cholesterol α-oxide was 2.7×10^6 dpm/mmole. Cholesterol β-oxide was synthesized by the method of Chicoye et al. (1968b) and purified by HPLC.

Glass-distilled, HPLC grade hexane, tetrahydrofuran, 2-propanol, chloroform, and acetone were purchased from Burdick and Jackson (Muskegon, MI) or Fisher Scientific Co. (Pittsburgh, PA). Methanol, ACS grade, was fractionally distilled after refluxing for 24 hr over magnesium. Trace water in solvents was removed using Molecular Seives 5A. All solvents were flushed with nitrogen before use.

Methods

Isolation of cholesterol oxides. Fifteen picograms of radioactive cholesterol α-oxide (approximately 8,000 cpm) in 15 μL chloroform, 16g egg yolk solid, and 300 mL acetone were blended in a Waring Blendor for approximately 15 sec. The mixture was filtered through a PTFE membrane (Millipore Corp., Bedford, MA) using an all-glass filter apparatus. The precipitate was extracted with an additional 300 mL acetone. After the combined filtrate was evaporated to dryness under reduced pressure, the solid was dissolved in 5 mL hexane containing 1% tetrahydrofuran. The extract was chromatographed by a silicic acid column [80g Bio-Sil HA (Bio-Rad Laboratory, Richmond, CA), 2.5 cm i.d. × 40 cm H] which had been equilibrated with hexane containing 1% tetrahydrofuran. The mobile phase, at 1 mL/min, changed linearly from the initial composition, 1%

tetrahydrofuran in hexane, to pure tetrahydrofuran in 1600 min. The eluent, collected in 20 mL fractions, was monitored for radioactivity using a Packard Liquid Scintillation Spectrometer (Packard, Model 3255, Downers Grove, IL). The fractions containing radioactivity were pooled, dried under nitrogen, and dissolved in 2% 2-propanol in hexane. The isolates were further purified by HPLC (Water Associates, Model ALC/GPC 244) using a Lichrosorb Si-60 column [4 mm i.e. × 250 mm L, 10μ packing material (EM Laboratories, Inc., Elmsford, NY)] and isocratic elution, 2% 2-propanol in hexane at 1 ml/min. Column elution was monitored with a differential refractometer (Water Associates, Model 401). The lowest detectable cholesterol oxides by differential refractometer is 5μg.

Cholesterol α-oxide and its β-isomer were collected separately and characterized by spectroscopic techniques.
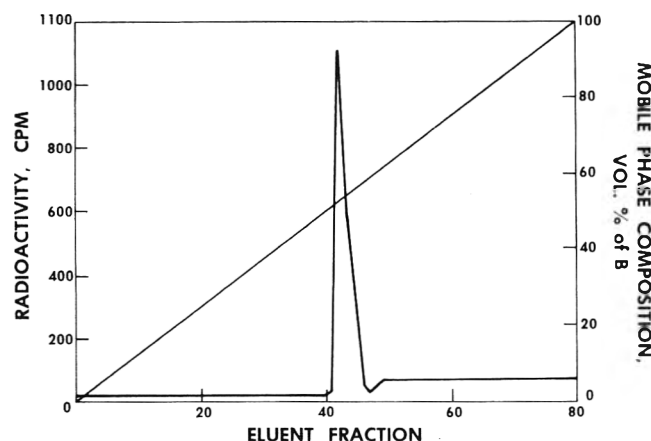
Fig. 1—Chromatograph of acetone extracts of egg yolk solids on a Bio-Sil HA column. Mobile phase changed linearly from 1% tetrahydrofuran in hexane to 100% tetrahydrofuran. Flow rate was 1.0 mL/min. Y axis represents the radioactivity of 1.0 mL aliquot of each 20 mL fraction.
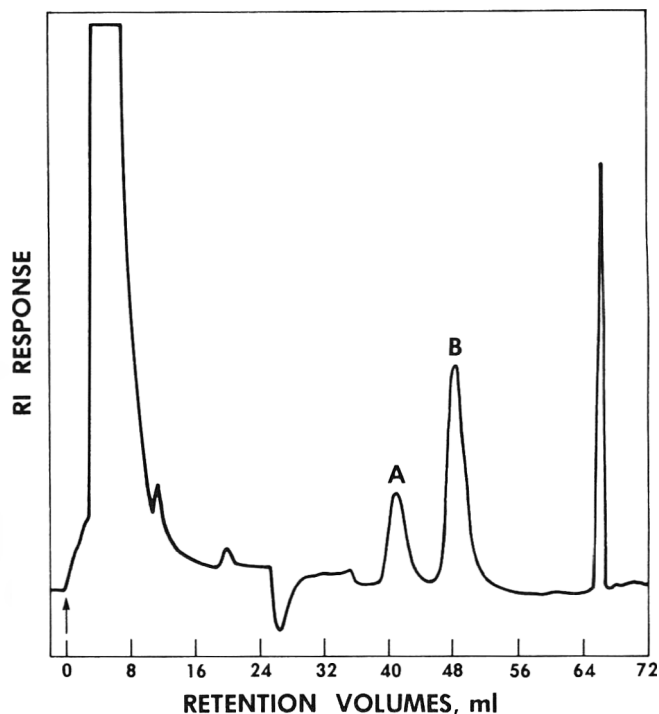
Fig. 2—HPLC of cholesterol oxide extracts of dehydrated egg yolk solids. Extracts, 75-90 μg, were eluted isocratically with 2% 2-propanol in hexane at 1.0 mL/min on a LiChrosorb Si-60 column and monitored by a differential refractometer.

Fig. 3—Mass spectra of (1) synthesized cholesterol α-oxide; (2) compound A; (3) synthesized cholesterol β-oxide; (4) compound B. Electron impact ionization of solid samples at 90°C and 70 ev.
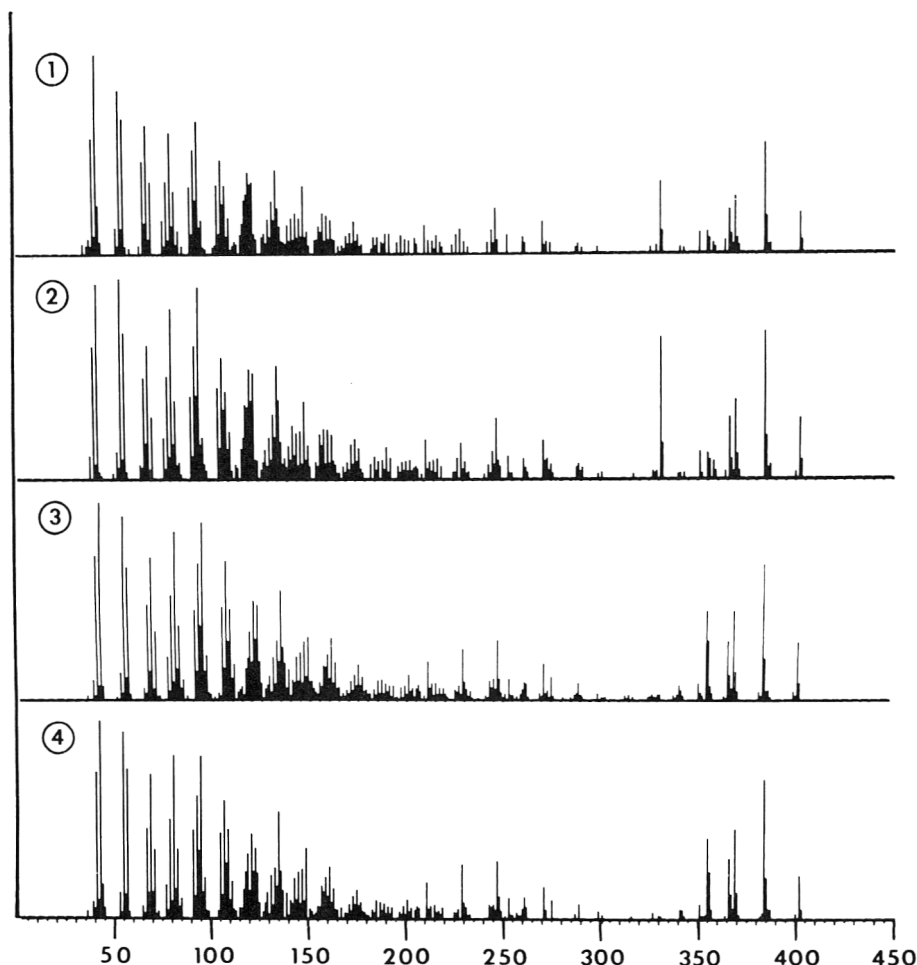
Mass Spectroscopy. Mass spectrum was obtained with a V.G. Micromass 70/70 F Mass Spectrometer (V.G. Analytical Ltd., Altrincham. Ches., England). Crystal samples were introduced directly on a solid probe and analyzed either with electron impact ionization or chemical (isobutane) ionization. The ion source temperature was 95°C and electron voltage 70 ev.

Infrared Spectroscopy. About 10–15 μL sample dissolved in carbon tetrachloride (66 μg/μL) was scanned with a Cary White-90 Infrared Spectrophotometer (Cary Instrument Co., Monrovia, CA) from 4000 to 400 cm^{-1}. The light path of the cell was 0.1 mm, and the slit width was set at 4 cm^{-1} at 4000 cm^{-1}.

Nuclear Magnetic Resonance Spectroscopy. Samples dissolved in deuterated chloroform were analyzed with a JEOL PFT-100 NMR Spectrometer (JEOL, Japan) at 99.5 MHz and room temperature. Proton NMR spectra were collected at 4-sec intervals and analyzed by Fourier Transform. The optimum number of scans was selected depending on the concentration of each sample.

RESULTS & DISCUSSION

ABOUT 60% of the yolk solids are lipids, approximately 4% of which are cholesterol. As minor compounds among the cholesterol oxidation products, cholesterol oxides are expected to be present in low concentration, if they are present at all. Acetone was chosen as the extracting solvent because it excludes phospholipids which constitute about

Fig. 4—Proton NMR spectra of (1) synthesized cholesterol α-oxide; (2) compound A; (3) synthesized cholesterol β-oxide; (4) compound B. Samples were dissolved in deuterated chloroform and analyzed with JEOL PFT-100 NMR Spectrometer at 99.5 MHz and room temperature. Spectra were collected at 4-sec intervals and analyzed by Fourier Transform.

Table 1—Proton NMR data of cholesterol oxides

| | Chemical shift, ppm | | | |
| | α-Oxide | | β-Oxide | |
	Synthesized	Isolated	Synthesized	Isolated
H-18	0.61[a](s)	0.63(s)	0.63(s)	0.63(s)
H-26/27	0.82(s)	0.83(s)	0.82(s)	0.82(s)
H-21	0.88(s)	0.90(s)	0.87(s)	0.89(s)
H-19	1.05(s)	1.08(s)	0.99(s)	0.99(s)
H-6	2.88(d)[b]	2.88(d)	3.05(s)	3.05(s)
H-3	3.89(b)[c]	3.87(b)	3.68(b)	3.68(b)

[a] singlet
[b] doublet
[c] broad

27% of the egg lipids. Acetone also allowed complete recovery of spiked radioactive cholesterol α-oxide. The chromatograph of crude acetone extract on a Bio-Sil column is shown in Fig. 1. Spiked radioactive cholesterol α-oxide was detected in fractions 40 through 47. The solvent composition in these fractions was 50–60% tetrahydrofuran in hexane.

Further chromatography of the isolated radioactive fraction using HPLC indicated that some egg samples contained compounds which coeluted with the spiked radioactive cholesterol oxides from the Bio-Sil column, and fresh liquid yolk, freeze-dried yolk and other collected samples contained an undetectable amount, less than 5 μg, of cholesterol α-oxide. Fig. 2 shows a typical HPLC chromatogram of an egg sample containing compounds coeluted with radioactive cholesterol α-oxide. Peak A was radioactive and had a relative retention volume (K′) of 12.5, which coincided with the K′ of synthesized cholesterol α-oxide. Peak B, K′ of 15.5, coincided with synthesized cholesterol β-oxide. Several mg of Compounds A and B were obtained by repeated HPLC separation.

The MS spectra of isolated Compounds A and B, Fig. 3, closely resemble those of synthesized cholesterol α- and β-oxides, respectively. This is not only strong evidence that Compounds A and B are cholesterol α- and β-oxides, respectively, it also indicates that they are free from impurities. The molecular weights of Compounds A and B as determined by electron impact and chemical (isobutane) ionization MS are 402, identical to cholesterol oxides. In addition to the parent ion M/z 402, the spectra show strong peaks of M/z 384 (M-H$_2$O), which is characteristic of 3β-hydroxy sterols, and of M/z 369 (M-H$_2$O-CH$_3$) which is also a well known feature of 3β-hydroxy sterols with angular methyl groups (McLafferty, 1980). Ion M/z 331 appears only in the spectra of the α-oxide and has been assigned to (M-C$_4$H$_7$O) as the result of cleaving bonds 1–10 and 4–5 on the A-ring. The configurational difference of the A/B junction between α-oxide (trans) and β-oxide(cis) accounts for the different amount of M/z 331 formed. The uniqueness of M/z 331 to the spectrum of α-oxide, may be used for quantifying α-oxide in a mixture of the two isomers.

The Proton NMR spectra of synthesized cholesterol α- and β-oxides and Compounds A and B are presented in Fig. 4. Peak 7.21 ppm, the reference peak, is the proton of deuterated solvent, chloroform. Peak 1.54 ppm represents the presence of trace water as contaminant. Sharp peak at 2.02 ppm in Part 4 is probably contamination from acetone. Major peaks were assigned by comparing them to the spectrum of cholesterol (Varian Associates, 1962) and to the work by Tori et al. (1964). The results, summarized in Table 1, show the close similarity of the spectra of the isolated Compounds A and B to those of their corresponding oxides. The most profound difference between the α- and β-oxides lies in the coupling of protons on C6 and C7. In α-oxide, C6 proton shows a distinctive doublet 4.0 cps, at
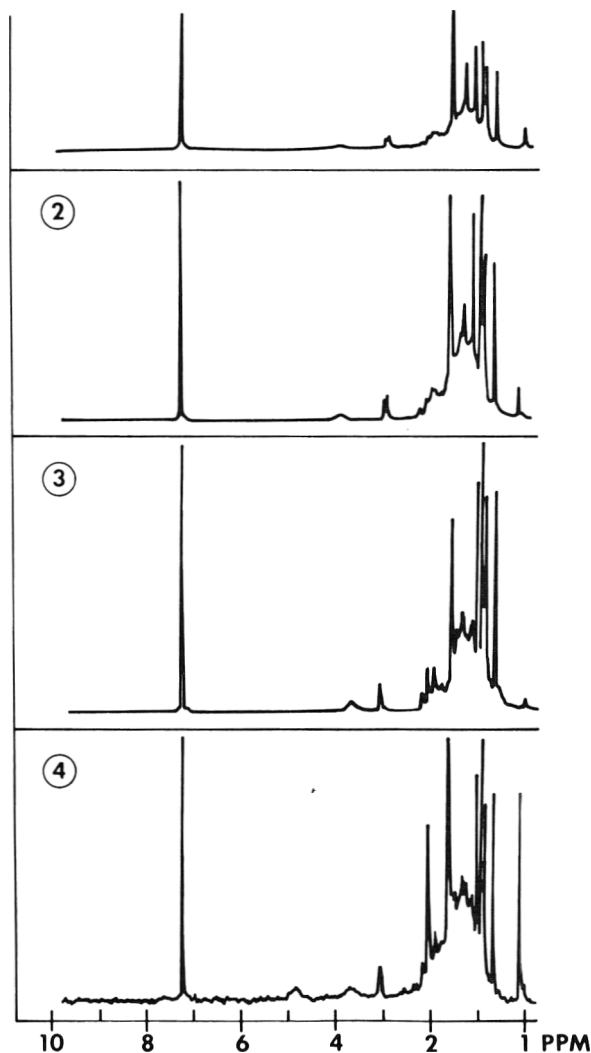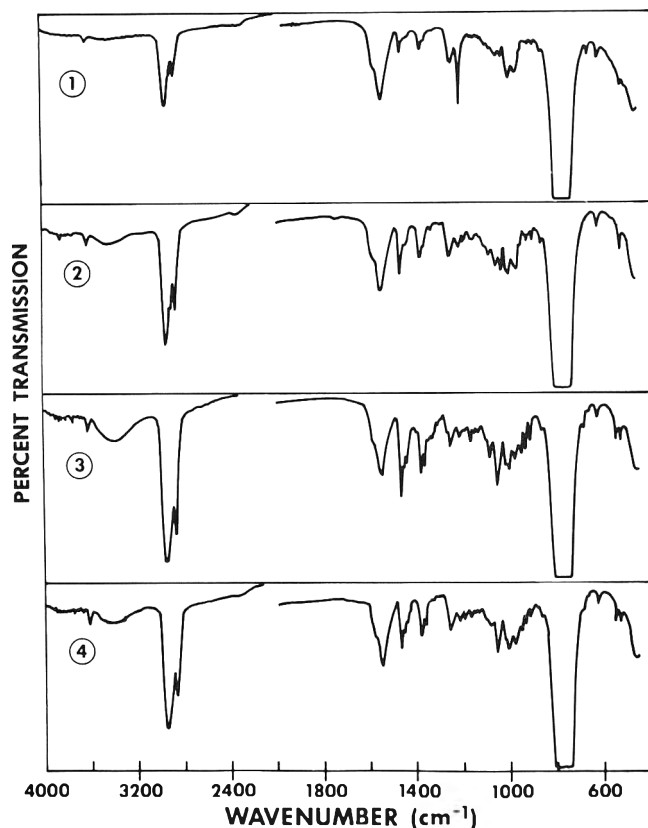
Fig. 5—IR spectra of (1) synthesized cholesterol α-oxide; (2) Compound A; (3) synthesized cholesterol β-oxide; (4) Compound B. About 15 μL sample in carbon tetrachloride was scanned with a Cary White-90 infrared spectrophotometer from 4000 to 400 cm⁻¹. The cell width was 0.1 mm, and the slit width was set at 4 cm⁻¹ at 4000 cm⁻¹.

2.88 ppm. The C6 proton in β-oxide shifts downfield to 3.05 ppm and the coupling is unresolved. The finding is in general gareement with the reported coupling constant of 3.8 cps for α-oxide and 2.5 cps for cholesterol β-oxide acetate by Tori et al. (1964). The configurational difference between the two isomers also results in different chemical shifts of the protons on C19 and C3, Table 1.

The IR spectra of Fig. 5 confirmed again the close similarity of Compound A to synthetic cholesterol α-oxide and Compound B to β-oxide. The unexpected peak at 1215 cm⁻¹ of Fig 5(1) is attributed to the contamination by chloroform which may have resulted from the incomplete evaporation of chloroform before the sample was dissolved in carbon tetrachloride. IR spectra show distinctively the presence of a single 3β-hydroxy group by the O−H stretch peak at 3610 cm⁻¹. There are also some apparent differences between α- and β-oxide in the fingerprint region. For example, in the region of 450–500 cm⁻¹ there is a single peak on the spectrum of α-oxide but a double peak on the spectrum of β-oxide. Generally speaking, IR offers limited usefulness for differentiating α- and β-oxide.

From the above MS, NMR and IR spectroscopic data, as well as the results of HPLC retention volumes, we have concluded that Compound A is cholesterol α-oxide and Compound B, β-oxide. The presence of cholesterol oxides in fresh commercially dehydrated whole eggs and egg yolks is believed to be induced by the drying process, since no oxides are found in fresh liquid eggs nor in the dry egg samples which were freeze-dried in the laboratory.

The average ratio of cholesterol α-oxide to β-oxide in egg products determined from the peak area of HPLC chromatograms (Fig. 2) is 1.0–2.7. This ratio is significantly higher

than that reported by Smith and Kulig (1975) on the oxidation of crystalline cholesterol (1 to 8-11) and slightly higher than we reported elsewhere (1-4) when a different isolation procedure was used (Tsai et al., 1980).

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Thermally Induced Gelation of Native and Modified Egg White-Rheological Changes During Processing; Final Strengths and Microstructures

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ABSTRACT

The heat-induced gelation of native egg white (EW) and egg white modified with succinic anhydride (SEW) or oleic acid (OEW), by addition of 15 moles of reagent/50000g protein, was evaluated. Rigidity modulus (G) and mechanical energy damping were continuously monitored during heating of the samples from 5 - 95°C in a nondestructive temperature-controlled thermal scanning rigidity monitor (TSRM). A measurable increase in G and decrease in energy damping were observed at lower temperatures for OEW than for EW. In SEW the measurable rheological transitions occurred at the highest temperature ranges. Failure strength of the cooked products (gels) evaluated using torsion and uniaxial compression tests revealed large differences due to treatments. Micrographs of gels showed apparent structural differences among treatments.

INTRODUCTION

HEAT-INDUCED PROTEIN GELS are generally the result of a transition from a viscous sol to a rigid and elastic solid-like structure. The mechanism involved in this transformation, however, has not been elucidated. Continuous measurement of an appropriate rheological property during heating presumably would yield valuable insight into the structure-development events in gel-forming proteins. This information would contribute to the understanding of the mechanism of protein gelation and should provide the basis for selecting adequate conditions for using proteins as texture-building components in heat-processed foods. Gossett et al. (1983b) developed a nondestructive method to continuously measure the force exerted by a gel on a wire probe connected to an electrobalance as the gelation process took place at a constant temperature. This force, however, was affected by thermal expansion of the gel and could not be used to directly measure transitions during continuous thermal processing of a gelling material. Montejano et al. (1983) reported a nondestructive technique for continuously monitoring modulus of rigidity and energy damping during heating of a minced fish paste (surimi). An instrument (Thermal Scanning Rigidity Monitor or TSRM) was developed for this purpose. Rigidity and energy damping values obtained with the TSRM were readily reproducible and were very sensitive in determining temperature ranges in which transitions occurred. The authors found good agreement between rigidity peaks and endotherm peaks from reported DSC studies of surimi. Burgarella (1983) modified the TSRM to enable the use of this instrument for a liquid to solid transition.

Since texture is one of the important quality attributes of a food product it might be expected that fundamental rheological characteristics of protein gel products would have been adequately studied. An examination of literature, however, reveals that basic rheological knowledge of protein gel systems is limited. To truly take advantage of the potential structure and texture building characteristics of gel-forming proteins one should be able, in addition to

monitoring changes during processing, to express the deformation, fracture and flow characteristics of final products in terms of fundamental rheological parameters (Hamann, 1983).

Egg white is widely used in the food industry because of its important functional properties. Chemical modification of egg white proteins has been carried out by several researchers to improve their biophysical and functional properties (Gandhi et al., 1968a,b; Sato and Nakamura, 1977; Ball and Winn, 1982; King et al., 1984) or to help in the study of protein gelation (Shimada and Matsushita, 1980; Ma and Holme, 1982). In a previous study (Montejano et al., 1984a) the mechanical failure characteristics of heat-induced gels from native egg white and egg white modified with acetic anhydride, succinic anhydride or oleic acid were evaluated. It was shown that oleic acid treated and succinylated egg white gels (25 moles reagent/50000g protein) had a significant increase in strength (shear stress) and deformability (shear strain) at failure as compared to native egg white gels. Therefore, it was decided to conduct the present research to study in more detail: (1) the changes in shear rigidity (stiffness) and elasticity (ability to spring back after deformation) during heating of native egg white and egg white modified with succinic anhydride or oleic acid; (2) the fundamental stress-strain conditions at structural failure of the heat-set gels; and (3) the ultrastructure of the gels by scanning electron microscopy (SEM).

MATERIALS & METHODS

Materials

Egg white was separated from the yolk of 1 day old eggs randomly collected from one strain of Single Comb White Leghorn laying hens (Poultry Science Dept., North Carolina State Univ., Raleigh, NC), thoroughly blended without foam formation, frozen, and held at −24°C until needed. All chemicals were analytical reagent grade.

Treatment of egg white

Prior to modification, egg white was thawed overnight in a refrigerator. Succinylation was performed by the method of Ball and Winn (1982) by the addition of 15 moles reagent/50000g protein. Treatment with oleic acid was performed as described by King et al. (1984) to a level of 15 moles reagent/50000g protein. The final pH for native and modified egg white was adjusted to 9.0 by the addition of 1N NaOH. This pH value was chosen not only because it is the common value in commercial practice but also because the strength of heat-induced egg white gels has been reported to be maximum at around this pH (Dunkerley and Zadow 1981; Hickson et al., (1980).

Preparation of heat-induced gels

Preparation of gels was as described by Montejano et al. (1984a) with changes. Briefly, samples were placed in stainless steel tubes (i.d. = 1.86 cm, L = 17.5 cm) coated with a thin film of Pam (Boyle-Midway, Inc., New York, NY) and tightly closed. In the present study the tubes were submerged in a water bath at 95°C. Heating was continued until the temperature in the center of the gels reached 90°C. The gels were immediately cooled in ice-water and shaped to the geometry required for mechanical failure testing. Gels were prepared in three replicates. At least 10 specimens for each type of test were obtained per replicate.

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Evaluation of shear rigidity and energy damping during cooking

Continuous measurement of the modulus of rigidity (G) and energy loss (energy damping) for native and modified egg white were performed in a Thermal Scanning Rigidity Monitor (Montejano et al., 1983) as modified by Burgarella (1983) to accommodate liquids. Fig. 1 shows the modified Thermal Scanning Rigidity Monitor (TSRM). Briefly, the TSRM consisted of a U-shaped jacketed chamber with two detachable and gasketed side walls, and a thin rectangular blade positioned in the center of the chamber. The chamber was fixed to the base of a Model 1122 Instron Universal Testing Machine (Instron Engineering Corp., Canton, MA). The center blade was fitted to a 50 kg compression load cell mounted in the crosshead of the machine. Liquid samples were poured into the chamber with side walls in position. A circulating water bath connected to the TSRM jacketed chamber provided controlled and uniform heating rates. Unless otherwise specified samples were heated at a constant rate of 0.5°C/min. Samples were heated from 5 – 95°C. Temperature inside the samples close to the jacketed wall was monitored by thin thermocouple probles inserted in each sample. At 2-min intervals a small cyclic force (from an upward-downward cyclic motion of the Instron's crosshead at 0.2 mm/min) was applied to the samples for two cycles to produce a constant peak to peak deformation in the sample of 4×10^{-5} m. Resulting peak to peak shear strain was 0.006. The force level was recorded on the Instron chart. Peak to peak forces ranged from 1×10^{-3} to 3.5×10^{-1} kg. Frictional force between samples and the removable side plates and the presence of a compressive force on the center blade caused by egg white material at the base of the chamber produced some deviaton from pure shear conditions in this device (Burgarella, 1983). In the present study the frictional and compressive forces (which are of measurable magnitude only when the samples start to form solid-like structures) were measured by removing the side plates and removing the gel formed on the bottom of the chamber. The decrease in the force level was of approximately the same proportion (about 50%) throughout the heating process after the formation of a solid-like structure and was applied as a correction producing G values more in agreement with the pure shear condition of the unmodified TSRM (Montejano et al., 1983). The modulus of rigidity (shear modulus) was calculated as the ratio of corrected peak shear stress to peak shear strain. Appropriate equations are given elsewhere (Burgarella, 1983; Montejano et al., 1984b). Apparent energy loss was calculated as the ratio of the hysteresis area divided by the work of deformation (Montejano et al., 1984b).

Ultimate strength in axial compression

Cylindrical compression specimens with a reduced diameter of 0.98 cm were cut from the center of the larger heat-induced gels using a cork borer. A length of 1.0 cm was used. Compression specimens were divided in half; one-half was used for evaluation of Poisson's ratio (lateral strain/axial strain) and the other half for axial compressive failure tests. Specimens at room temperature were compressed between flat parallel oil-lubricated plates mounted on the Instron machine. The crosshead speed was 10 cm/min producing a shear strain rate of 0.148 s^{-1}. Force and displacement at failure were recorded. True shear stress, true shear strain and shear modulus were calculated using the equations given by Hamann (1983). Poisson's ratio was assumed to be a constant and evaluated by compressing the specimens to no more than 10% axial deformation. The decrease in length of the compressed specimen was obtained from the force-deformation curve. The corresponding increase in diameter was measured at two different axial locations with a six-power measuring microscope and averaged. Barreling in the specimens was minimum at this axial deformation level.

Ultimate strength in torsion

The torsion failure tests were performed as described by Montejano et al. (1983). Cylindrical specimens from the heat-induced gels with a length of 2.87 cm were reduced in cross-section at the midsection to obtain a dumbbell shaped specimen. A minimum diameter of 1 cm was obtained at the midsection. The torsion apparatus was mounted on the Instron testing machine. The right

Fig. 1—Thermal scanning rigidity monitor for liquids.
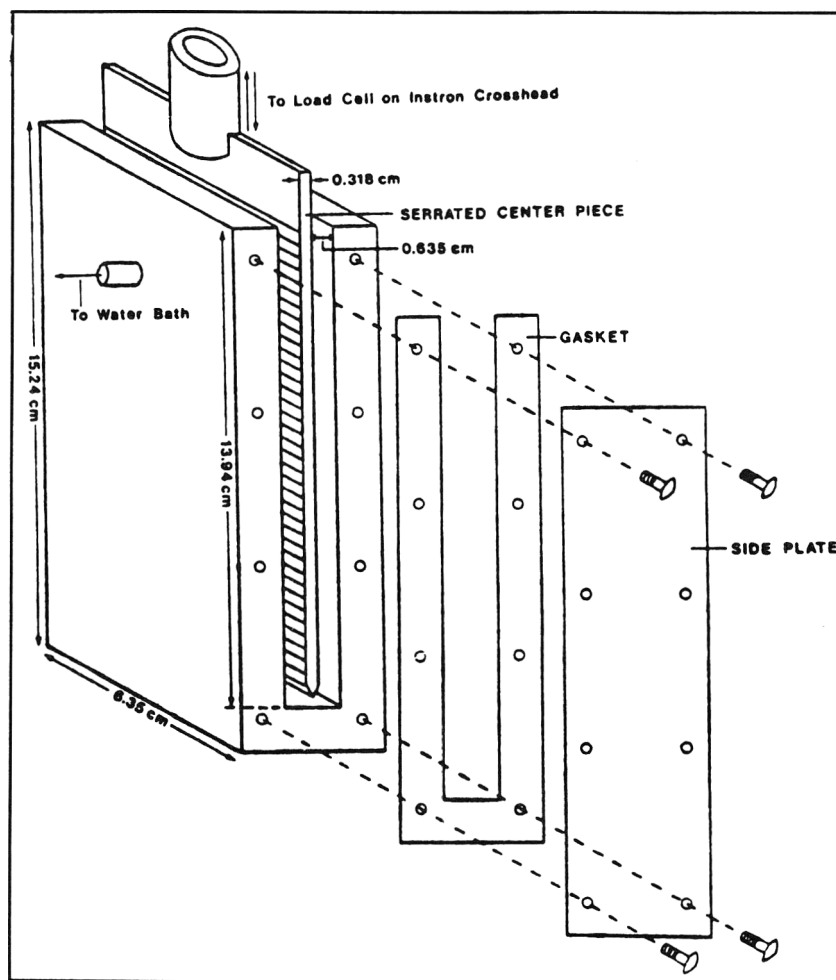
end of the specimen was fixed while the left end was rotated about its axis. Specimens were tested at room temperature. Crosshead speed was 20 cm/min (twisting speed = 1.675 rpm) and shear strain rate was 0.113 s^{-1}. Shear stress, true shear strain and shear modulus were calculated using the equations given by Hamann (1983).

Microstructure

Samples were prepared for SEM by pre-fixation of rectangular strips (15 × 5 × 5 mm) of heat-induced gel in 4% glutaraldehyde (in 0.1M phosphate buffer) for 2 hr at 2°C. The strips were then submerged in liquid nitrogen to produce rapid freezing. The frozen samples were fractured and cut to a smaller rectangular size (2 × 2 × 0.5 mm). The opposite side of the cryo-fractured gel surface was marked with a fine-point marker. The specimens were immediately thawed and fixed in 4% glutaraldehyde (in 0.1M phosphate buffer) for 2 hr at 2°C. The fixed specimens were rinsed twice with 0.1M phosphate buffer. The next step was dehydration through a graded series of ethanol (30, 50, 70, 95, 100% ethanol in water for 15 min each), followed by a graded series of Freon 113 (30, 50, 70, 95, 100% Freon 113 in ethanol for 15 min each). The dehydrated specimens were critical-point dried in a Bomar SPC-1500 (The Bomar Co., Tacoma, WA) critical point dryer using Freon 113 as the transitional fluid. The dried specimens were mounted on aluminum studs with conductive colloidal graphite exposing the cryofractured surface and coated with palladium/gold by diode sputtering in a Hummer V (Technics Ems. Inc., Springfield, VA) sputter coater to a thickness of 30 nm, measured by a digital thickness monitor E1A-416 (Technics, Inc., Springfield VA). Microstructure was observed with a Jeol JSM-T200 scanning electron microscope at a voltage of 15 kV. At least five specimens of each material were observed for each one of the three preparations and a large number of micrographs were taken at magnifications ranging from 500 to 15000X in order to select truly representative micrographs.

Statistical analysis

Data were analyzed using analysis of variance and comparison of treatment differences by Scheffe's S statistic (John, 1971).
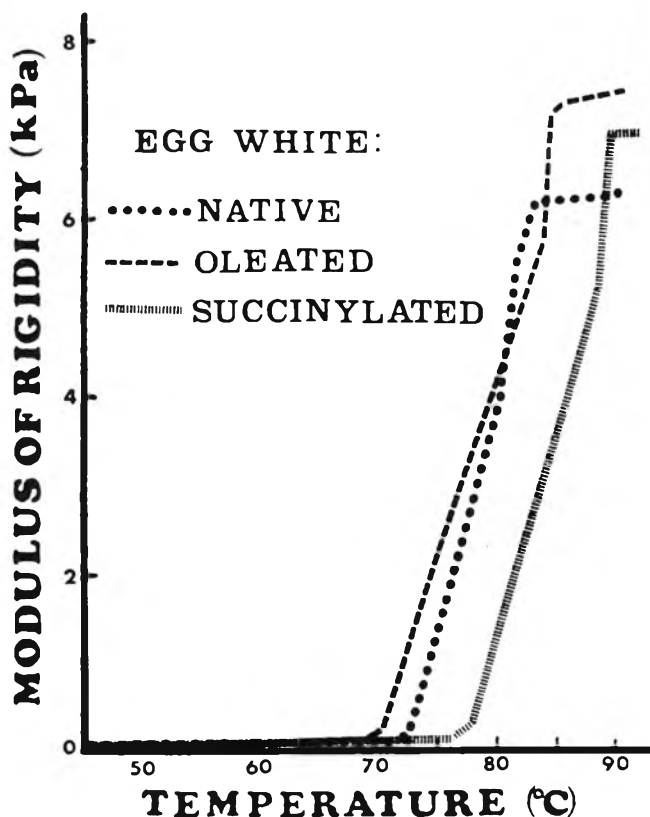
RESULTS

Rigidity scanning

Plots of modulus of rigidity (G) versus internal temperature for native and modified egg white are shown in Fig. 2. Low rigidity (stiffness) values for all materials were observed below 60°C because they exhibited fluid behavior. Native egg white (EW) showed a rapid increase in rigidity starting at 71°C and leveling off at 83°C. It was noticed that between 80 and 82°C the increase in rigidity was steepest. Oleic acid treated egg white (OEW) showed a rapid increase in rigidity starting at a lower temperature (68°C) as compared to EW. The rate of increase was less than that of EW. A steeper increase in rigidity was observed between 84 and 85°C and leveling off occurred at 85°C. The final rigidity value for OEW (7.32 kPa) was higher than that of EW (6.26 kPa). For SEW the development or rigidity occurred at the highest temperature range (76 – 91°C). The rate of increase was similar to that of OEW. As with the other materials a steeper increase in rigidity was noticed at the upper end (89 – 91°C). SEW presented a final rigidity value (7.02 kPa) between those of EW and OEW. The above results indicate that chemical modification had an effect on the coagulation temperature of egg white proteins and in the stiffness of the final heat-set product. All the plots, however, presented the same general pattern.

Elasticity scanning

Monitoring of apparent energy loss (energy damping) was employed as an inverse measure of change in elasticity (ability to spring back after deformation) during heating. Fig. 3 shows plots of apparent energy loss versus internal temperature for native and modified egg white. In general, all the materials exhibited a transition from a viscous fluid

Fig. 2—Shear rigidity thermograms for native and modified egg white. Heating rate = 0.5°C/min.
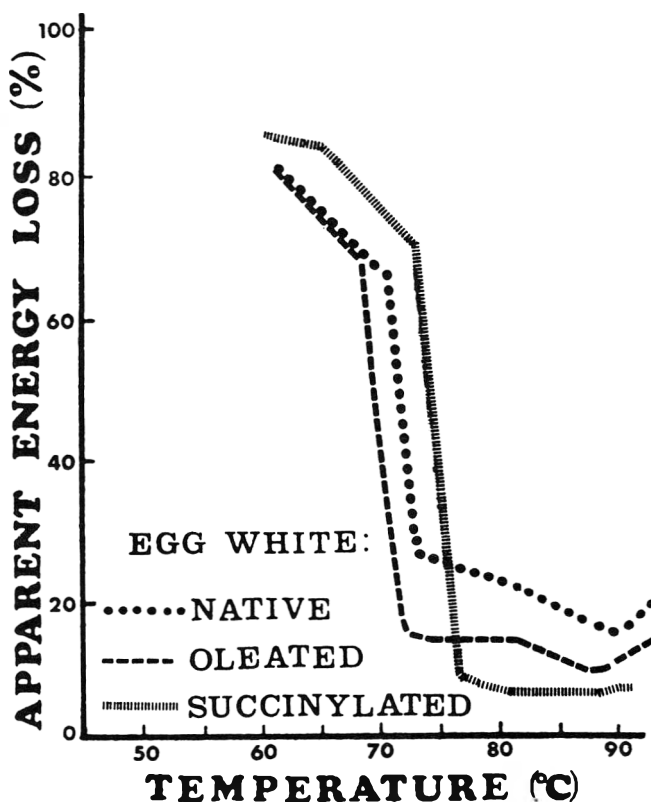
Fig. 3—Energy loss thermograms for native and modified egg white. Heating rate = 0.5°C/min.

to an elastic solid structure. During a loading-unloading cycle an ideal (Newtonian) fluid would have a 100% energy damping (no shape recovery) while an ideal elastic (Hookean) solid would present a 0% energy damping (complete shape recovery). Starting at around 61°C all the materials presented a measurable and moderate decrease in energy loss. This initial decrease was followed by a steep linear decrease in the loss of energy for all the materials, indicating that structures capable of more rapid shape recovery were being developed. In the case of EW, energy loss decreased from 67 to 25% in the 70 – 74°C temperature range. For OEW the rapid decrease in energy loss from 68 to 15% was observed at a lower temperature range (68 – 73°C). In SEW the rapid decrease in energy loss from 71 to 10% occurred at the highest temperature range (74 – 78°C). These observations indicate that chemical modification had an effect on the development of an elastic structure during heating. After the steep decrease, EW still showed a small decrease in energy loss in the 74 – 89°C range reaching a 15% energy loss. This was followed by a slight increase to 19% at the high temperature end (89 – 91°C). OEW presented approximately uniform values after the steep decrease in energy loss although minor transitions were observed at 81 and 87°C. The final energy loss value (13%) for OEW was smaller than that of EW. More uniform values were presented by SEW following the steep decrease in energy loss reaching a final value of 6%. The above observations indicate that a fairly elastic structure was obtained for each material over a short temperature span and further temperature increase had either a small or negligible effect upon it.

Poisson's ratio

An average Poisson's ratio value of 0.49 was obtained tor each material, (Montejano, 1983). Poisson's ratio values are required for calculating true shear stress and true shear strain at failure from force and displacement at failure in axial compression (Hamann, 1983).

Stress and strain at structural failure

Table 1 lists the mean values of the mechanical failure parameters obtained from torsion and compression tests of EW and OEW. Failure in compression did not occur in gels made from SEW. For EW true shear stress, true shear strain and shear modulus values were not significantly different when comparing torsion and compression failure results in agreement with previous findings (Montejano et al., 1984a). For OEW, however, all the compression failure parameters were significantly different than those from torsion failure tests. In order to produce failure in compression for OEW gels the samples required an average true axial strain of 93.5%. Thus, the samples underwent gross deformation before failure occurred.

Torsion tests produced failure in gels from both native and modified egg white. Torque versus angle of twist graphs were approximately linear to the failure point indicating a nearly linear elastic behavior to failure in the gels. Table 2 lists the mean values of the failure parameters from torsional testing of native and modified egg white gels. Inspection of this table shows that gels from both OEW and SEW had higher strengths, as revealed by shear stresses at failure, and were more ductile as revealed by larger true shear strains at failure when compared to EW. Gels from OEW had shear stress values at failure almost twice, and true snear strains about 1.5 times as large as those of EW. Gels from SEW had the highest values of shear stress and true shear strain at failure being about 2.5 and twice as large, respectively, as compared to those of EW. Visual observation

EGG WHITE:

(A) NATIVE
(B) OLEATED
(C) SUCCINYLATED

5 μm
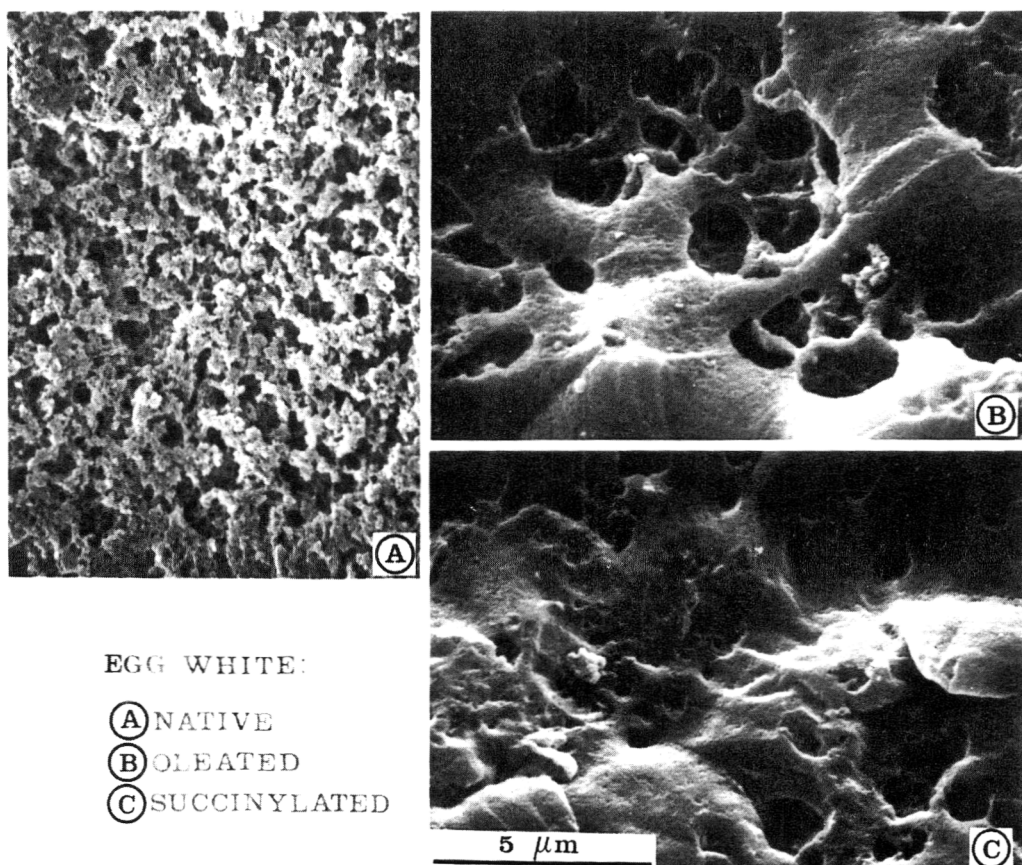
Fig. 4—SEM micrographs of cryofractured surfaces of heat-induced gels from native and modified egg white. Magnification = 5000X.

of the gel specimens after failure showed that torsion specimens from all the materials failed at approximately a 45° angle from the long axis of the specimen. This indicates torsion failure occurred mainly in a tension mode (Hamann, 1983).

Microstructure

The ultrastructures of cryofractured surfaces from native and modified egg white gels at a magnification of 5000X are shown in Fig. 4. EW formed a three dimensional sponge-like network of relatively small aggregates. Such a protein network structure seems suitable for trapping water. A similar structure for egg white has been reported by several researchers (see for example Siegel et al., 1979). At the same magnification level both OEW and SEW presented structures different from that of EW. A matrix of dense protein surrounding large open pockets in which water was likely enclosed was observed in both gels. A somewhat tighter structure was observed in SEW than OEW.

DISCUSSION

Egg white proteins

The first measurable decrease in energy loss observed at around 61°C for native and modified egg white (Fig. 3) could be expected to be associated with the initial stages of protein denaturation and the formation of aggregates. At pH values around 9, the initial denaturation temperature of egg white proteins has been reported by several researchers to be in the 58 – 62°C range (Payawal et al., 1946; Slosberg et al., 1948; Siedman et al., 1963; Cunningham, 1977). Johnson and Zabik (1981), studying denaturation temperatures of albumen proteins singly and in combination in a custard model system, found that the control mixture (with a composition similar to that of native egg white) at pH 8.0 presented a transition in the 61.5 – 62.5°C range. The authors attributed this transition to denaturation of conalbumin and possibly other protein(s). Donovan et al. (1975) using differential scanning calorimetry (DSC) at a heating rate of 10°C/min found that at pH 9 the thermogram of conalbumin had an endotherm peak at 62°C. Lineweaver et al. (1967) estimated that heating egg white at pH 9 for 3.5 min at 62°C would alter more than 50% of the conalbumin. Thus it is very likely that the initiation of the elastic structure at 61°C is related to denaturation of the least heat stable albumen protein, conalbumin.

Donovan et al. (1975) reported denaturation temperatures of 72.5 and 77°C for lysozyme and ovomucoid, respectively, while Johnson and Zabik (1981) found denaturation temperatures of 71.5 and 72°C for ovalbumin and albumen globulins, respectively. Matsuda et al. (1981) reported that the ovalbumin-aggregation temperature in egg white covers a temperature span from 70 – 76°C at pH 7

and 9. It is possible, therefore, that the rapid decrease in energy loss for egg white in the 70 – 74°C range (Fig. 3) was subsequent to denaturation of several of the minor albumen proteins and the beginning of denaturation for the major albumen protein, ovalbumin; and their participation in an aggregation process leading to a gel network formation. Since ovalbumin comprises more than 50% of the albumen (Powrie, 1977) a large contribution to the elasticity and rigidity of the system would be expected from its denaturation and aggregation.

The rapid increase in rigidity for EW starting at approximately 71°C (Fig. 2) indicated that the three-dimension network was increased with further energy input. It is interesting to note that above 74°C the decrease in energy loss (Fig. 3) for EW was very small but that rigidity was increasing at a rapid rate (Fig. 2). It is possible that the albumen gel was developed over a short temperature span coinciding with the relatively short temperature span for denaturation of egg white proteins. This fortuitously allowed what could be considered to be a coordinated participation of the various albumen proteins in an aggregation process necessary for the formation of an elastic structure. Once the elastic three dimensional network was set, further heating promoted completion of the aggregation step (additional bonds) resulting in the increase in rigidity but having only a slight effect on the elasticity of the structure. A similar behavior was observed in thermally-induced minced fish muscle gels by Montejano et al. (1983).

The steeper increase in rigidity between 80 and 82°C for EW (Fig. 2) may have been related to further changes in ovalbumin. DSC data using heating rates 20 times those applied in this study yielded denaturation temperatures for ovalbumin ranging from 79 – 84°C (Hegg et al., 1979; Donovan et al., 1975; Karmas and DiMarco, 1970) which suggest that completion of the denaturation process and aggregation of ovalbumin could be responsible for the rapid increase in rigidity above 80°C. In the context of this discussion it must be mentioned that heating rate, in general, has an effect on the observed response in thermal studies of protein systems. It has been reported that as the heating rate increases the magnitude of the indicator scoring parameter (such as heat flow, rigidity, etc.) becomes moderately smaller and occurs at slightly higher temperatures (Montejano et al., 1983; Donovan et al., 1975). Varying heating rates from 0.5 – 2.0°C/min, however, did not have a significant effect on rate of change in energy loss during heating of minced fish muscle (Montejano et al., 1983). Limited studies with EW using a heating rate of 2.0°C/min produced an apparent energy loss versus temperature plot that was not significantly different from that reported here. The modulus of rigidity versus temperature plot, however, presented a curve that was shifted towards higher temperatures by approximately a constant factor of 4°C and at any given temperature the rigidity was lower by

Table 1—Mean values of mechanical failure parameters from torsion and axial compression tests[a]

| Material | True shear stress | | True shear strain | |
	Torsion (kPa)	Comp. (kPa)	Torsion	Comp.
Egg White (Native)	12.61[c] (2.21)[b]	13.24[c] (2.40)	1.21[c] (0.12)	1.27[c] (0.21)
Egg White (Oleated)	22.78[c] (3.77)	39.37[d] (13.19)	1.72[c] (0.16)	1.87[d] (0.23)

[a] Means of 30 observations.
[b] Values given in parentheses represent standard deviations.
[c,d] The same letter superscripts within each material indicate that the associated numbers in the two columns are not significantly different (P > 0.05).

Table 2—Mean values of torsional failure parameters for native and modified egg white gels[a]

Material	Shear Stress (kPa)	True Shear Strain
Native Egg White (EW)	12.61[c] (2.21)[b]	1.21[c] (0.12)
Oleic Acid Treated EW	22.78[d] (3.77)	1.72[d] (0.16)
Succinylated EW	30.97[e] (7.17)	2.40[e] (0.24)

[a] Means of 30 observations.
[b] Values given in parenthesis represent standard deviations.
[c,d,e] Means within a column with different superscripts are significantly different (P < 0.05).

about 1.8 kPa. Since the use of high heating rates allow less time at any given temperature for the proteins to complete denaturation and aggregation it appears that the formation of an elastic structure would be the result of the initial aggregation of denatured proteins. On the other hand, rigidity (which is influenced by rate of temperature increase) would be dependent on a higher degree of unfolding and ordered aggregation which would increase the stiffness of the system. By using different heating rates, thereby producing different levels of unfolding and aggregation it should be possible to obtain structures with the same elasticity but different rigidities.

Succinylated egg white

The delay in the occurrence of the rheological transitions in SEW (Fig. 2 and 3) may be due to the changes in charge characteristics associated with modification. Succinylation shifts isoelectric points to a lower pH and results in a net gain of two negative charges on the modified protein (Ball and Winn, 1982; Brekke and Eisele, 1981; Ma and Holme, 1982). The increase in negative charge causes electrostatic repulsions that must be overcome before the formation of aggregates (Habeeb et al., 1958; Nakamura et al., 1978). Improved heat stability of SEW has been reported by several researchers (Ball and Winn, 1982; Ma and Holme, 1982). Groninger (1973) repoted that aqueous disperions of 1 – 2% succinylated myofibrillar fish proteins did not coagulate or precipitate when heated at 100°C.

Ma and Holme (1982) observed that succinylation of egg white did not significantly alter the configuration of the proteins. Furthermore, they found that the surface hydrophobicity of egg white remained virtually constant following succinylation. Palladino (1980) did find that succinylation increased the molecular radius of ovalbumin and that there was a greater exposure of hydrophobic amino acid residues. Thus, the first measurable decrease in energy loss in SEW (Fig. 3), which occurred at about the same temperature as in EW (61°C) may be an indication of initial protein denaturation. A higher degree of denaturation and unfolding apparently occurred in the succinylated proteins before aggregation as evidenced by the very slow rate of decrease in energy loss between 61 and 65°C (Fig. 3). Additionally, between 74 and 78°C SEW presented a rapid decrease in energy loss; however, significant increase in rigidity did not occur until about 76°C and at 78°C it was still small. Therefore it appears that a very elastic but weak structure was obtained as a result of limited and partial aggregation of the denatured proteins.

Several researchers have reported that the formation of disulfide bridges during heating is essential in egg white proteins and other proteins for the development of aggregates (Jensen, 1959; Huggins et al., 1951; Hawler, 1954; Tombs 1970; Pour-el and Swenson, 1976). Van Kleef et al. (1978) treated ovalbumin with a concentrated urea solution (6M) in an attempt to obtain heat-set gels in which only disulfide bridges were present. Mechanical evaluation of the ovalbumin/urea gels showed that they possessed a high reversible deformability (i.e. elasticity) and a low viscous character indicating a rubber-like behavior. In addition to disulfide bond formation, noncovalent attractive forces such as hydrophobic and electrostatic interactions have been reported to be involved in thermally induced protein gelation. Ma and Holme (1982) observed a large increase in surface hydrophobicity in both native and succinylated egg white following heat treatment. The authors suggested that hydrophobic interactions are directly involved in gel formation. Shimada and Matsushita (1980) arrived at the same conclusion from studies on thermal coagulation of native and succinylated egg albumin

(80% ovalbumin, 20% conalbumin). Egelandsdal (1980), however, concluded that thermal gelation of ovalbumin was governed primarily by electrostatic forces. The high final elasticity and rigidity presented by SEW (Fig. 2 and 3) may be attributed to more protein denaturation before aggregates were formed. Further research is needed to directly relate the role of disulfide bonds and noncovalent interactions to the development of rigidity and elasticity in the structures.

Oleated egg white

In OEW the initial rheological transitions were observed at the lowest temperatures (Fig. 2 and 3). It has been reported that treatment of egg white with oleic acid results in an increase of the overall negative charge of the proteins (King et al., 1984). It would be expected, then, that electrostatic repulsions among protein molecules be increased and thermocoagulation retarded, which is not the initial observed response. In contrast to Palladino's (1980) results with succinylated egg white, physiochemical examinations of OEW (King et al., 1984) did not reveal any significant alterations of protein structure. Viscosity and other data did suggest that lipid coated, mixed protein micelles were formed. It is very difficult to speculate on the mechanisms of denaturation and aggregation for such a system. The results reported here (Fig. 2 and 3) do suggest that the protein was disposed to denaturation at lower temperatures and that the denatured protein aggregated in a manner favoring the formation of very elastic and very rigid gels with characteristics similar to gels formed from SEW. Additionally, present evidence suggests that hydrophobic interactions are mainly involved in the binding of large organic anions, such as fatty acids, to globular proteins with little participation of the carboxylate group (Spector, 1975). Gelation temperatures of various muscle proteins have been shown to decrease following treatment with arylating reagents which introduce hydrophobic groups on the surface of the proteins (Niwa et al., 1981a, b).

Measurable decrease in energy loss for OEW was observed at about the same temperature as the rapid increase in rigidity (Fig. 2) indicating that aggregates were being formed along with the elastic structure. As with the other materials the decrease in energy loss occurred over a shorter temperature span as compared to the increase in rigidity. The slower rate of increase in rigidity of OEW as compared to EW (Fig. 2) suggests that as more thermal denaturation and unfolding occurred in the proteins electrostatic repulsions increased at a faster rate than the attractive forces. This delay in the increase in rigidity presumably may be the cause of the increase in coagulation temperature of crude ovalbumin treated with anionic detergents reported by Hegg and Lofqvist (1974). The final higher rigidity value may indicate a larger extent of protein denaturation and unfolding producing strong aggregates.

Micrographs

A typical scanning electron micrograph of EW (Fig 4) shows the presence of more or less spherical particles, suggesting that aggregation of the proteins occurred randcnly. It is believed that in complex globular protein systems, such as egg white, aggregation may occur randomly and simultaneously with protein denaturation, forming structure composed of strands in which spherical aggregated particles would predominate (Tombs, 1970, 1974; Schmidt, 1981). Native egg white gels seem to have a large number of channels available for water to flow through. Freezing would create large channels due to ice crystal formation explaining

the poor freeze-thaw response (Davis et al., 1952). The microstructures presented by both OEW and SEW (Fig. 4) show a very dense primary structure with little space for binding water. On the outer boundaries of the aggregates it may be possible to bind large amounts of water due to the presence of large open pockets in the structures. Similarly, Simonsky and Stanley (1982) observed that SEM micrographs of extrudates from succinylated soy proteins presented dense structures surrounding large open cells. Both SEW and OEW yielded translucent heat-induced gels while EW gels were opaque. OEW produced gels more opaque than SEW but much less than EW. The degree of opaqueness in these gels seems to be directly related to the total quantity of refractive surfaces presented by their structures.

A high net charge repulsion between proteins, limited intermolecular interactions and a low dry matter content have been suggested as conditions required for the formation of translucent gels (Hegg et al., 1979; Hegg, 1982; Egelandsdal, 1980). The present results, however, indicate that molecular interactions were present in the translucent gels producing areas of dense aggregates. It appears that the increase in the net negative charge of the proteins delayed and/or lowered the rate of protein aggregation allowing for more denaturation and unfolding. The particular configuration and spatial arrangement of the protein-water aggregates gave the translucent characteristics to the structure. Higher final rigidity and lower final energy loss values were observed in both SEW and OEW as compared to EW (Fig. 2 and 3). Furthermore, inspection of Table 2 reveals that in the cooked product higher shear stress at failure and higher true shear strain at failure were required in both SEW and OEW as compared to EW, indicating that the latter was both weaker and less deformable. Several researchers have observed that opaque gels from globular proteins are more fragile and brittle than translucent gels (Shimada and Matsushita, 1980; Nakamura et al., 1982; Egelandsdal, 1980).

An added functional advantage of SEW and OEW gels is the observed freeze-thaw stability (Ball and Winn, 1981; King et al., 1984; Gossett and Baker, 1983; Gossett et al., 1983). One reason for this stability may be that water is strongly trapped or bound in the gels. Upon cooling of the gels, hydrogen bond formation will contribute to the immobilization of water, thus ice crystal formation during freezing is minimized due to a high degree of water immobilization preventing damage to the structure and syneresis (Parducci and Duckworth, 1972). Additionally, it would be expected that the presence of a strong impermeable and deformable matrix that encloses the water pockets will withstand ice crystal formation without appreciable damage.

Poisson's ratio and gel strength

The average Poisson's ratio value obtained for the heat-induced gel from each material (0.49) was in agreement with the values reported by Montejano et al. (1984a) for gels from native egg white and egg white modified with succinic anydride or oleic acid (25 moles of reagent per 5×10^4 g EW protein). Poisson's ratio is a measure of bulk compressibility of a material. As Poisson's ratio approaches 0.5 a homogeneous isotropic material exhibits incompressibility and the amount of trapped gas within it is small. The heat-induced gels tested in this study are, therefore, nearly incompressible. Chemical modification did not seem to have an effect on the volume of trapped gas in the gels.

Evaluation of the mechanical failure characteristics of heat-induced gels from EW, OEW and SEW indicated that, due to the high deformability and strength of the modified EW gels, compressive failure testing had only limited application. The disagreement between torsion and compression failure parameters (Table 1) can be at-

tributed to the gross deformation in shape of the OEW gels required for failure to occur. Monteiano et al. (1984a) observed that gels prepared from succinylated or oleic acid treated albumen (25 moles reagent per 5×10^4 g EW protein) were highly deformable and did not fail in uniaxial compression testing. Previous results from our laboratory have shown a good agreement between "true" compressive and "true" torsion failure parameters for a variety of materials when axial compressive deformation was less than 85% (Montejano et al., 1984a, b; Diehl et al., 1979). Torsional testing, on the other hand, has the ability to produce failure even in highly deformable materials without geometry alteration effects. Additionally, a nearly elastic behavior to failure was observed in all the gels tested in the present study. The same nearly-elastic behavior has been observed in heat-induced gels from minced fish (Montejano et al., 1983; 1984b) pork, beef and turkey (Montejano et al., 1984b) even though, in general, they required large true shear strains at failure. Similarly, Diehl et al. (1979) found a nearly linear torsion response with apple flesh but a much lower true shear strain at failure than the heat induced gels mentioned above. Thus, it is believed that torsional testing yields reliable values in failure testing of heat-induced gels.

It is common to take the initial tangent of the compressive force-deformation curve as a measure of gel strength. Low strain force-deformation response is not a measure of breaking strength, however. It is a measure of rigidity. Hermansson (1982) observed that the initial tangent of force-compression curves for heat-induced plasma protein gels did not change when the pH was varied from 6 to 10 even though structural changes were evident. At pH 6 and 7 compressive failure was observed at different force and deformation values. Wood (1979) compared the initial tangent and the peak (failure) force from compression testing of a series of seven polysaccharide gels. A different order of ranking was obtained for the gels from the two measurements. It is, therefore, evident that care should be taken in selecting a method to measure the rheological characteristics of gel systems.

Comparisons of shear moduli obtained from compression, torsion and TSRM tests are shown in Table 3. Initial shear moduli from compression tests ranked the materials in a different order than any of the other measurements. The lower values of G observed for OEW and SEW indicate that these materials presented a behavior similar to a soft rubber in that they deformed very easily initially; however, large force and deformation were required for failure to occur. Since the heat-induced gels presented a highly elastic behavior (Fig. 3) there was a good agreement between initial and failure G values from torsion tests. It is interesting to notice that for EW, a more brittle material, there was no significant difference in G values between torsion and compression tests. Lower final G values for all materials were obtained from TSRM evaluations although the ranking order was the same as in torsion. This was because G values from the TSRM were determined at a high temperature while those in torsion were evaluated at room temperature. Higher values would be expected at lowered temperatures due to hydrogen bond formation during cooling increasing the rigidity of the gels. In comparing G values from torsion and the TSRM it is interesting to notice that the ratio of the TSRM value to the torsion value was approximately the same magnitude for all three materials.

Influence of final cooking temperature on SEW gels

Mechanical failure evaluation of heat-induced gels of native and modified egg white using different concentrations of reagents (25M for OEW and 15 and 25M for SEW) and a lower final temperature (80°C) has been previously reported (Montejano et al., 1984a). It was ob-

Table 3—Comparison of shear moduli from different tests for native and modified egg white gels[a]

Material	Initial G[b] (Compression) (kPa)	Initial G[b] (Torsion) (kPa)	G at failure[c] (Torsion) (kPa)	G @ 90°C from TSRM (kPa)
EW	9.17[d] (1.35)	9.67[d] (1.96)	9.88[d] (1.88)	6.26[e] (0.93)
OEW	7.44[d] (1.16)	11.99[e] (1.36)	11.70[e] (1.58)	7.32[d] (0.70)
SEW	5.85[d] (0.89)	10.39[e] (2.09)	10.22[e] (2.34)	7.02[f] (0.57)

[a] Values given in parenthesis represent standard deviations.
[b] From initial slope of force-deformation curves.
[c] Ratio of shear stress/shear strain at failure.
[d,e,f] The same letter superscripts within each material (line) indicate that the associated numbers are not significantly different (P > 0.05).

Table 4—Comparison of torsional failure parameters for succinylated egg white cooked to different final temperatures[a]

Material[1]	Shear Stress (kPa)	True Shear Strain
SEW (80°C)[b]	14.88[d] (3.09)[c]	2.49[d] (0.204)
SEW (85°C)	21.44[e] (6.57)	2.41[d] (0.371)
SEW (90°C)	30.97[f] (7.17)	2.40[d] (0.24)

[a] Final temperature in center of gel given in parenthesis.
[b] From Montejano et al. (1984a)
[c] Values given in parenthesis represent standard deviation.
[d,e,f] Means within a column with different superscripts are significantly different (P < 0.05)

served that for SEW an increase in concentration of reagent produced a significant increase in shear stress at failure but did not affect the true shear strain at failure. The values reported, however, were lower even at the higher concentration level than the ones found for 90°C in the present study. Observation of Fig. 2 shows that for SEW there is a large increase in rigidity from 80 - 90°C. For EW and OEW the increase in rigidity above 80°C is not as large. Failure parameters for EW at 80°C (Montejano et al., 1984a) and 90°C (Table 2) are not significantly different. Gels prepared from OEW to a final temperature of 80°C had only slightly lower values for both shear stress and true shear strain at failure than the gels at 90°C. To further study the effect of final temperature SEW gels were cooked to a final temperature of 85°C and the value of the failure parameters were compared to those at 80°C (Montejano et al., 1984a) and 90°C. The results are tabulated in Table 4. There was a large increase in shear stress at failure but no significant difference in true shear strain at failure as final temperature in the gels increased. These results indicate that above 80°C it is possible to obtain gels from SEW with a deformability (true shear strain) at failure almost double that of EW and various degrees of strength (shear stress) as a function of temperature. At 80°C the shear stress at failure of SEW gels is not significantly different from that of EW (Montejano et al., 1984a) but at 90°C there is a significant difference by a factor of about 2.5. Thus, textural characteristics of EW can be significantly altered by means of chemical modifications and processing temperatures.

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Effect of Pre-Processing Storage Conditions on the Composition, Microstructure, and Acceptance of Sweet Potato Patties

W. M. WALTER, JR. and M. W. HOOVER

―――――――――――ABSTRACT―――――――――――

'Jewel' and 'Centennial' sweet potatoes were processed into frozen patties at harvest, after curing 1 wk, and after curing and storing up to 26 wk. Sugar, starch, and pectin contents were determined on raw roots, cooked slices, and finished patties. The starch content after cooking was correlated with consumer acceptance and is the most important factor in the preparation of a patty of consistent quality from both fresh and stored roots. Scanning electron microscopy showed that in the cooked, pureed sweet potatoes most of the cells had been ruptured and that the patty was held together by an amorphous matrix consisting of added ingredients and spilled cellular contents.

INTRODUCTION

SWEET POTATOES in the form of pre-cooked, frozen patties are presently manufactured only during the harvest season since stored roots undergo internal changes which make production of a patty of consistent quality difficult. The nature of these changes is due in part to the increase in activity during storage of amylolytic enzymes (Walter et al., 1975). These enzymes cause starch breakdown during cooking and thereby strongly influence the textural properties and flavor of the processed product. It is likely that other as yet undefined microstructural changes mediated by storage may also affect the flavor and texture of the final product.

An earlier paper described the ingredients and processing conditions necessary to produce consumer acceptable patties from freshly harvested, cured, and cured and stored 'Jewel' and 'Centennial' sweet potatoes (Hoover et al., 1983). The present study was undertaken to evaluate the effect of processing steps and storage history on the carbohydrate content and microstructure of sweet potatoes and sweet potato patties.

MATERIALS & METHODS

Sweet potato patties

The patties were prepared from 'Jewel' and 'Centennial' sweet potatoes which were freshly harvested, cured 1 wk at 32°C and 80-90% relative humidity (RH), and cured roots held for up to 6 months at 13-16°C and 80-90% RH (Wilson et al., 1980). The patty preparation process was as described by Hoover et al. (1983). Briefly, the peeled, sliced roots were cooked 5 min in steam in a continuous cooker, blended with the other ingredients, comminuted in a hammer mill, and finish cooked in a steam injector (steam at 160°C) flow system. The cooked puree was then vacuum cooled to 68°C, molded into patties, and then frozen at −20°C. When all of the stored roots had been processed, patties were removed in batches from the freezer and cooked 2 min in a deep fat cooker in peanut oil at 171°C before sensory evaluation. Each batch of patties was prepared from 4.53 kg cooked potatoes, 381g cornstarch, 453g sucrose, 45.4g mono- and diglyceride mixture, 13.6g sodium chlo-

ride, 2.3g sodium acid pyrophosphate, and 0.7g FD & C yellow No. 6.

Analysis

Samples (in duplicate) were taken from the raw sweet potato strips, cooked strips, and patties. Dry matter, sugar and starch content, alcohol-insoluble solids, and pectin content were determined. In addition, oil absorption was measured on deep fat fried patties. Dry matter content also was measured on the comminuted mixture.

Sugars

Ten-gram samples were blended with 50 mL 95% ethanol and 8 mL water for 1 min. The mixture was transferred to a 100-mL volumetric flask and held for 1 wk at room temperature. One-milliliter aliquots were placed in 20 mL vials and lyophilized. Before analysis, each vial was derivitized (Oupadissakoon et al., 1979), and 2-μL samples were injected into a Packard model 800 gas-liquid chromatograph (GLC). Separation was carried out on a 1.82 m x 6.4 mm i.d. glass column packed with 3% OV-17 on 100/120 Chromosorb W. Carrier gas (N_2) flow rate was 30 mL/min. The injector was at 240°C and detector at 300°C. Peaks were detected with a flame ionization detector. The column oven was held at an initial temperature of 200°C for 8 min and then programmed at 25°C/min to 290°C. A Hewlett Packard model 3390A integrator operating in the internal standard mode was used for integration. The integrator was calibrated daily using duplicate standards containing fructose, glucose, sucrose, and maltose at levels near those found in the samples.

Alcohol-insoluble solids

Twenty-five gram samples were blended 1 min with 150 mL 80% ethanol. The solution was clarified by centrifugation and the liquid phase discarded. This procedure was repeated using 250 and 150 mL boiling 80% ethanol and 0.5 min blending times for the second and third extractions. The final residue was removed, put into a tared weighing dish, air dried at room temperature overnight, dried at 95°C for 24 hr in a forced draft oven, cooled, and reweighed. The alcohol-insoluble solids (AIS) was calculated on the basis of fresh sample weight.

Starch

From 12-15 mg alcohol-insoluble solids were weighed into 50 mL Erlenmeyer flasks, 5 mL distilled water added, and the samples heated to 121°C in an autoclave. After cooling, 1 mL 0.01M acetate (pH 4.6) and 0.01M NaCl buffer containing 2 mg amyloglucosidase (*Rhizopus* mold; 5,000-10,000 units/g) and 2 mg α-amylase (*Bacillus subtilis*; 1,400 units/g) were added to each sample. The samples were incubated at 50°C for 1 hr. Fifteen milliliters acetate-NaCl buffer were added to bring the pH to 4.6, and the samples were incubated at 50°C for 2 hr. The solution was transferred to a 25-mL volumetric flask. The glucose content was measured on a suitable aliquot using the glucose oxidase procedure (Dekker and Richards, 1971). The starch content was calculated from the amount of glucose released.

Pectins

Pectin content, expressed as hexuronic acids, was determined on 0.1g samples alcohol-insoluble solids as described by Scott (1979). Galacturonic acid was used as a standard. The method described above was evaluated using laboratory samples containing 80% starch, 16% cellulose and 4% pectin to simulate the alcohol-insoluble solids fraction. The starch and cellulose were shown to cause no significant interference.

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Microscopy

Tissue was fixed in 3% glutaraldehyde, dehydrated, critical point dried, gold-coated, and photographed at various magnifications at 20 KeV in an ETEC Autoscan microscope (Walter and Schadel, 1982).

Fat absorption

'Jewel' patties were dried in a forced-draft oven overnight at 60°C, followed by 4 hr at 98°C. The patties were crumbled and extracted overnight with boiling hexane:ether (1:1). The solvent was evaporated and the amount of fat was determined. Fat absorption was calculated by subtracting the fat content before frying from the fat content after frying.

Statistical analysis

Statistically significant differences (P ≤ 0.05) in the amount of each compositional component (i.e. sugars, starch, pectin, etc.) over time were determined by a one-way analysis of variance procedure and the Waller-Duncan K-ratio 't' test (SAS, 1982).

RESULTS & DISCUSSION

FOR BOTH CULTIVARS, as length of storage increased, starch content decreased and sugar levels increased (Fig. 1 and 2), reflecting root metabolic activity. In addition, fructose and glucose content increased more rapidly in 'Jewel' than in 'Centennial' during storage. Sucrose levels increased during storage in 'Centennial' more than in 'Jewel' until the 16-wk sampling date. The total sugar content, however, was similar for both varieties at each sampling date. No maltose was detected in raw roots.

When the sliced roots were cooked, no change occurred in the content of sucrose and glucose plus fructose (Fig. 1 and 2). The apparent decrease in these sugars for cooked roots can be explained by slight changes in dry matter. The same pattern was found for both cultivars.

Cooking caused a significant decrease in starch content and attendant maltose formation in both cultivars, which reflected heat-mediated enzymatic conversion of the starch (Fig. 1 and 2). The maltose content and starch remaining after cooking decreased with length of storage of the raw roots prior to cooking. However, the percentage of starch converted during cooking was dependent upon the cultivar. For 'Jewel' the percentage of starch converted by cooking decreased from 38% at harvest to 26% after roots had been stored for 6 months (Table 1). 'Centennial' root starch was converted during cooking at about the same rate (33-40%) regardless of the length of storage prior to cooking. This is unlike baked roots in which the starch conversion percentage increased from around 65% at harvest to more than 90% after 2 months of storage (Walter et al., 1975).

It has been demonstrated (Deobald et al., 1969; Walter et al., 1975) that α-amylase activity increased during storage, and thus, starch conversion would be expected to increase with length of storage due to increased enzymatic activity. This was observed for baked roots (Table 1), but

Table 1—Percentage of starch converted[a] during cooking of sweet potatoes

Time after harvest	Cooked strips		Baked, whole
	'Jewel'	'Centennial'	'Centennial'[b]
0 (at harvest)	38.8	38.2	65
1 wk	42.5	36.0	—
6 wk	39.0	37.6	75
2 months	37.3	32.6	95
4 months	30.6	35.7	—[c]
6 months	25.6	39.9	—[c]

[a] Amount of starch in raw sample — amount of starch in cooked sample ÷ amount of starch in raw sample x 100.
[b] Data from Walter et al. (1975).
[c] Experiment conducted for 2 months.
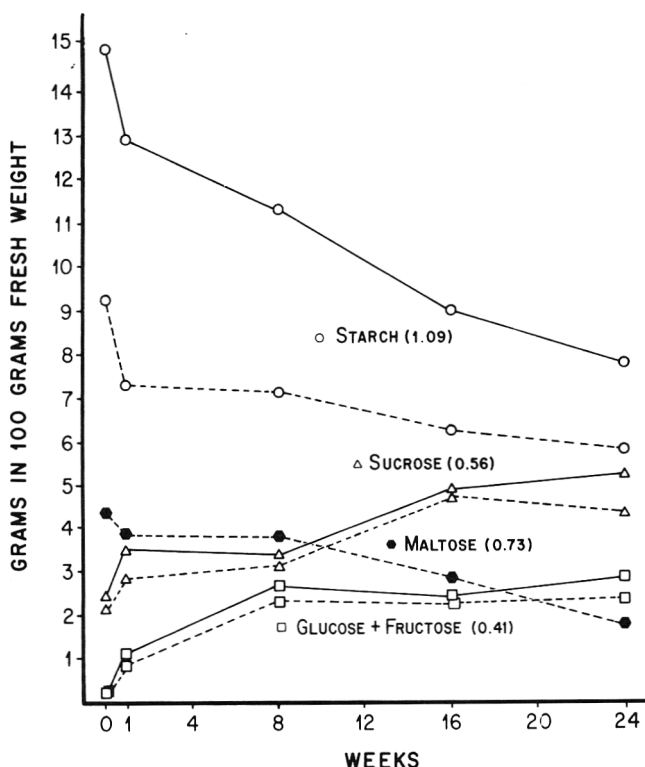
Fig. 1—Starch, ○ (1.09); sucrose, △ (0.56); fructose plus glucose, □ (0.41); and maltose, • (0.73) content for raw (——) and cooked (---) 'Jewel' cultivar sweet potatoes. Numbers in parentheses are least significant differences (P ≤ 0.05). Maltose was not detected in raw sweet potatoes.
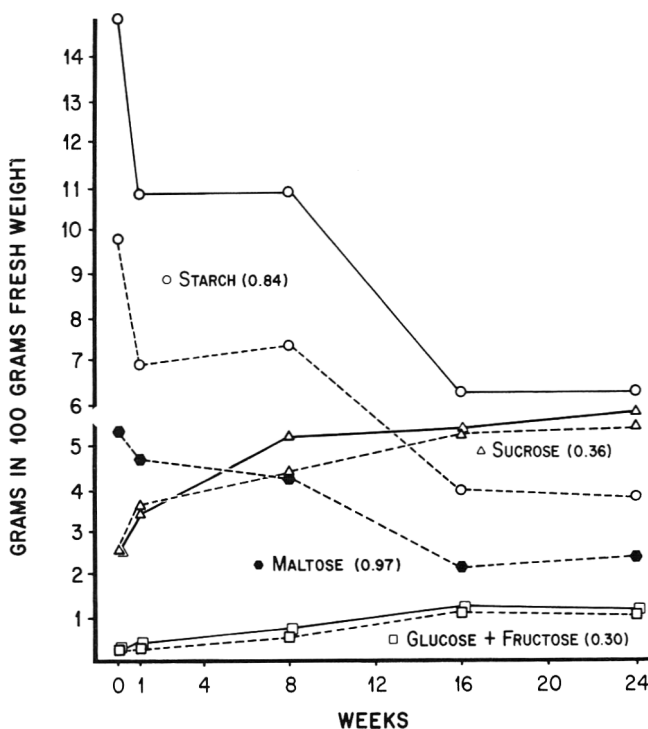
Fig. 2—Starch, ○ (0.84); sucrose △ (0.36); fructose plus glucose, □ (0.30); and maltose, • (0.97) content for raw (——) and cooked (---) 'Centennial' cultivar sweet potatoes. Numbers in parentheses are least significant differences (P ≤ 0.05). Maltose was not detected in raw sweet potatoes.
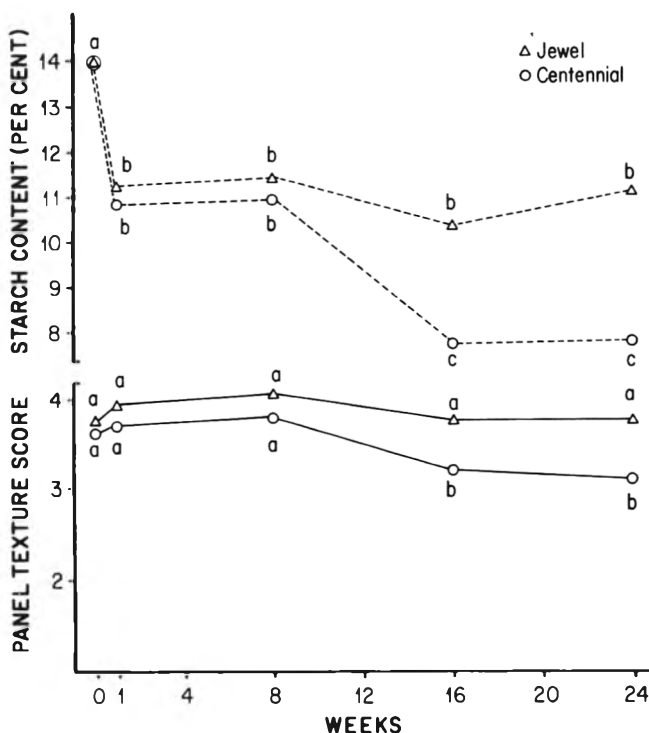
Fig. 3—Comparison of starch content of sweet potato patties and taste panel texture score data from Hoover et al. (1983) for 'Jewel' and 'Centennial' patties. Data points with different letters are different at $P \leqslant 0.05$.

not cooked strips. Probably the cooking procedure produced such a rapid temperature increase in the strips that the enzymes were inactivated before extensive starch conversion could occur. Thus, starch conversion and maltose formation exhibited an apparent dependence on starch content.

Since the goal of producing a consistent product requires that the starch content be constant, it would be advantageous to use cultivars such as 'Jewel' in which the percent starch conversion decreased with storage. In such a situation, starch added at a constant level yielded a finished product with similar starch levels, except for the zero time sample (Table 2). In contrast, 'Centennial' patties showed a decreasing level of starch over time due to a constant percentage of starch conversion. These differences in patty starch content were reflected by the taste panel data (Hoover et al., 1983; Fig. 3). 'Jewel' patties had a constant texture score declined significantly after the 2-month sample, thus, paralleling the decline in starch content. The total sugar content of patties (Table 2) for both cultivars was fairly constant, and included the natural sugars, the maltose formed during cooking, and the sucrose added after cooking. Pectin levels in patties for both cultivars fluctuated with no definite trend.

Scanning electron microscopy

Examination of the SEM photomicrographs revealed that cooking caused some cells to assume a wrinkled, crumpled appearance together with cellular separation (Fig. 4A and 4B), but with little cell rupture. Starch remained compartmentalized within the cells and, therefore, was not available to bond the cells together.

Fig. 4—Scanning electron photomicrographs of cooked, 'Jewel' sweet potato slices. (A) cured 1 wk, bar = 100μ (B) cured 1 wk, bar = 10μ

Table 2—Starch, total sugar and pectin content[a] of sweet potato-based patties[b]

Time after harvest	Starch		Total sugar		Pectins	
	'Jewel'	'Centennial'	'Jewel'	'Centennial'	'Jewel'	'Centennial'
0 time	14.10[a]	14.10[a]	10.15[b]	11.38[a]	0.45[b,c]	0.47[b]
1 wk	11.20[b,c]	10.72[b]	11.36[a]	10.53[a]	0.50[b]	0.56[a]
2 months	11.41[b]	10.89[b]	11.50[a]	10.84[a]	0.43[c]	0.45[b,c]
4 months	10.15[c]	8.25[c]	12.21[a]	11.19[a]	0.81[a]	0.45[b,c]
6 months	11.08[b,c]	8.38[c]	12.08[a]	11.73[a]	0.29[d]	0.42[c]

[a] Grams in 100g of patty.
[b] Means within columns followed by the same letter are not significantly different at the 5% level.

Fig. 5—Scanning electron photomicrographs of puree prepared from cooked, 'Jewel' strips plus added ingredients. (A) bar = 100μ. (B) bar = 10μ.

The step after cooking involved addition of starch, sugar, and other ingredients, followed by pureeing in a hammer mill. Photomicrographs (Fig. 5A and 5B) of this material showed that the added material was attached to the surface of the cells, perhaps bonded by the cellular starch released by the shearing action of the hammer mill. Steam injection of the puree completed the cooking process. In the steam injected material, cells were bonded together by a formless material (Fig. 6) with only the cell outlines visible. This amorphous, glue-like material provided the rigidity necessary to retain the patty shape.

Fat absorption

When patties were fried, the 0 time and 1 wk samples absorbed more oil than did patties prepared from roots stored for longer periods. The fat content (corrected for fat present before frying) was 3.2 and 5.7% ('Jewel' and 'Centennial,' respectively) for patties prepared from freshly harvested roots and ca. 7% (both cultivars) for patties prepared from roots cured 1 wk. The fat content of patties prepared from roots stored 2 months or longer ranged from 1.3–2.8%. There was no correlation between the amount of oil absorbed during cooking and the sensory panel ratings (Hoover et al., 1983), indicating that oil absorption was not a factor in panel acceptance.

Fig. 6—Scanning electron photomicrograph of sweet potato patties prepared from 'Jewel' puree. Bar = 10μ.

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Optimization of Nutrient Retention During Processing: Ascorbic Acid in Potato Dehydration

MARTIN MISHKIN, ISRAEL SAGUY, and MARCUS KAREL

―――――――――ABSTRACT―――――――――

A kinetic model representing ascorbic acid degradation in potatoes as a function of moisture content and temperature was used to find optimal dryer-temperature control paths for minimizing ascorbic acid loss during air drying of potato disks. Optimal dryer-temperature control was also determined for minimizing drying time given a specified minimum retention of ascorbic acid. Constraints were placed on the final moisture content and the air temperature. Optimization was achieved using a simulation-optimization approach based on the complex method.

INTRODUCTION

THE USE OF dynamic optimization procedures in food processing operations in general has been limited, and this is true in particular of dehydration. Several attempts have been made to optimize drying processes with respect to minimizing drying time and/or energy costs (Thompson, 1967; Thygeson and Grossman, 1970; Farmer, 1972; Brook, 1977; Doe and Menary, 1979; Bertin and Srour, 1980; Militzer, 1981, 1982b, 1982c). However, there are few published accounts of maximizing product quality attributes such as color or nutrient retention.

Karel (1975) has reviewed the fundamentals of air drying. There are a multitude of drying schemes whereby a vegetable product, for example, could be dried to a specified moisture content. The air flow rate and wet- and dry-bulb temperatures may be varied during drying as well as the shape, size, orientation and loading of the vegetable product. In this study we are reporting techniques for finding the optimal dryer temperature control path for maximizing ascorbic acid retention and for minimizing drying time with a constraint on resultant ascorbic acid retention.

MODELS

Kinetic model for ascorbic acd

The kinetic model representative of ascorbic acid degradation during air-drying of white potato disks was obtained using a dynamic test approach (Saguy et al., 1978; Mishkin, 1983; Mishkin et al., 1982). An empirical first-order kinetic model was used.

$$-dC/dt = kC \qquad (1)$$

where C is the concentration of ascorbic acid (normalized with respect to initial concentration). The first-order rate constant (k) has Arrhenius temperature dependence,

$$k = k_o \exp[-E_a/RT] \qquad (2)$$

where k_o and E_a have moisture functionality,

$$\ln k_o = P_1 + P_2 M + P_3 M^2 \qquad (3)$$

$$E_a = P_4 + P_5 M + P_6 M^2 + P_7 M^3 \qquad (4)$$

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and where M is the moisture content in g/g-solids. The moisture and temperature dependence of the rate constant associated with this model, for the parameter estimates (Table 1), is illustrated in Fig. 1. The moisture dependence of E_a is shown in Fig. 2.

Moisture model

Fig. 3 shows the initial shape and size of the potato disks used for simulation. These dimensions correspond to the initial dimensions, i.e. before shrinkage occurs. Moisture transfer during drying was described by Fick's Law for unsteady unidirectional diffusion,
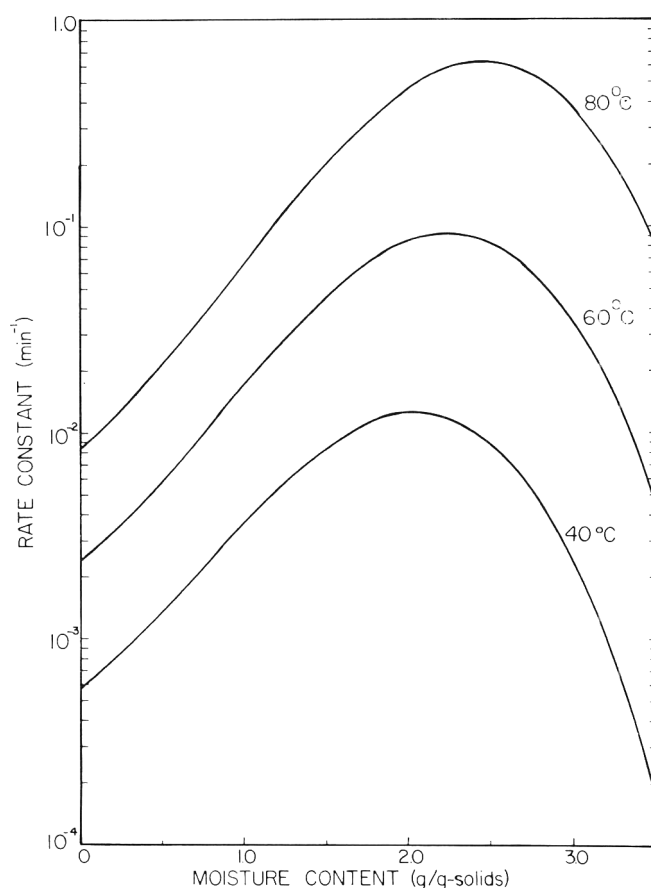
Fig. 1―Moisture and temperature dependence of the first order reaction rate constant.

Table 1―Ascorbic acid kinetic model parameter values for Eq. 3 and 4 (Mishkin, 1983)

Parameter	Value
P_1	16.38
P_2	1.782
P_3	1.890
P_4	14831.0
P_5	241.1
P_6	656.2
P_7	236.8

$$\frac{\partial M}{\partial t} = \frac{\partial}{\partial x}(D \frac{\partial M}{\partial x}) \qquad (5)$$

where D is the effective diffusion coefficient (cm^2/sec). Edge effects were ignored due to the high ratio of diameter to thickness of the potato disks. Temperature dependence of the diffusion coefficient (D) was expressed by an Arrhenius expression,

$$D = D_{ref} \exp [\frac{-E_D}{R}(\frac{1}{T} - \frac{1}{T_{ref}})] \qquad (6)$$

where $T_{ref} = 313°K$. D_{ref} and E_D have moisture dependence expressed by Eq. 7 and 8 (Luyben et al., 1980):

$$D_{ref} = \exp [-\frac{P'_5 + P'_6M}{1 + P'_7M}] \qquad (7)$$

$$E_D = \frac{P'_8 + P'_9M}{1 + P'_{10}M} \qquad (8)$$

Eq. 5 has been approximated using a finite difference approach. Fig. 4 shows the initial nodal format. For the interior nodes the differential equation describing the i-th volume element is Eq. 9:

$$\frac{dM_i}{dt} \cong \frac{D_i}{(\delta x_i)^2}[M_{i+1} - 2M_i + M_{i-1}] \qquad (9)$$

Eq. 9 was derived assuming that the bulk solids density is approximately uniform in the region represented by "Z" in Fig. 4. It is also assumed that D is nearly uniform in this small region if the nodes are close enough. Shrinkage is taken into account by assuming shrinkage proportional to decrease in moisture concentration (Mishkin, 1983),

$$\delta x_i = \delta x_0 [P'_3 \frac{M_i}{M_0} + P'_4] \qquad (10)$$

where δx_0 is the initial distance between adjacent nodes, M_0 is the initial moisture content, and P'_3 and P'_4 are parameters estimated from drying data ($P'_3 = 0.35$, $P'_4 = 0.65$) (Mishkin, 1983). Based on order of magnitude analysis the surface volume element is assumed to remain at the equilibrium moisture content (M_{eq}). Drying occurs identically from both sides of the potato disk, thus only half the disk must be modeled, due to the symmetry of the problem. Therefore, for the centerplane node,

$$\frac{dM_N}{dt} \cong \frac{-D_N}{2(\delta x_N)^2}[M_N - M_{N-1}] \qquad (11)$$

where

$$\delta x_N = 1/2 \, \delta x_0 [P_3 \frac{M_N}{M_0} + P'4] \qquad (12)$$

and where N is the number of volume elements used in the finite difference scheme. N was reduced to the lowest level possible without compromising accuracy, by trial and error (N = 10). The average moisture content (M) is represented by Eq. 13:

$$M = \frac{(M_1 + M_N)/2 + \sum_{i=2}^{N-1} M_i}{N - 1} \qquad (13)$$

Estimated parameter values for Eq. 7 and 8 are listed in Table 2 (Mishkin, 1983). For drying simulation M_{eq} was estimated using sorption data from Goerling (1958).

Temperature model

The temperature of the potato disks (T_s) was modelled using an algebraic approximation to the heat balance (Mishkin, 1983; Mishkin et al., 1982) where the temperature was assumed uniform,

$$T_s = \frac{\lambda_w m_s}{hA} \frac{(dM)}{dt} + T_{db} \qquad (14)$$

where m_s is the mass of solids within the disk, (dM/dt) is the rate of vaporization of water, T_{db} is the air temperature and λ_w is the latent heat of vaporization for water. Temperature uniformity was verified experimentally by inserting fine thermocouple probes at various depths within potato disks during drying. Air-exposed
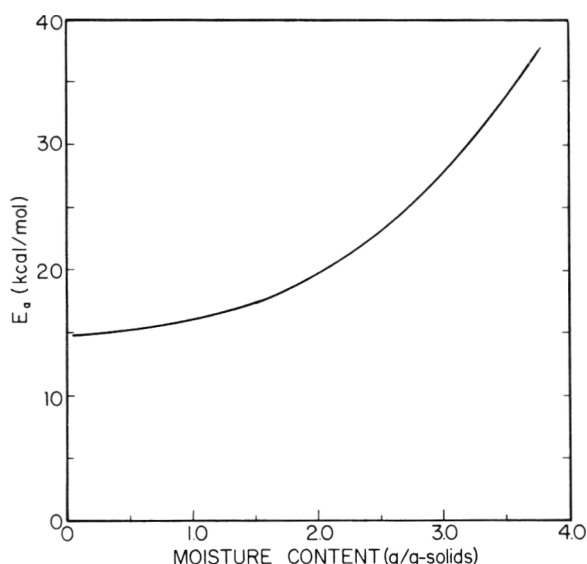
Fig. 2—Moisture dependence of activation energy.
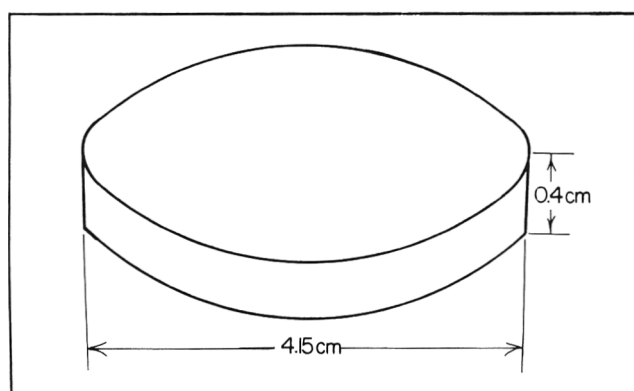
Fig. 3—Initial dimensions of potato disks.
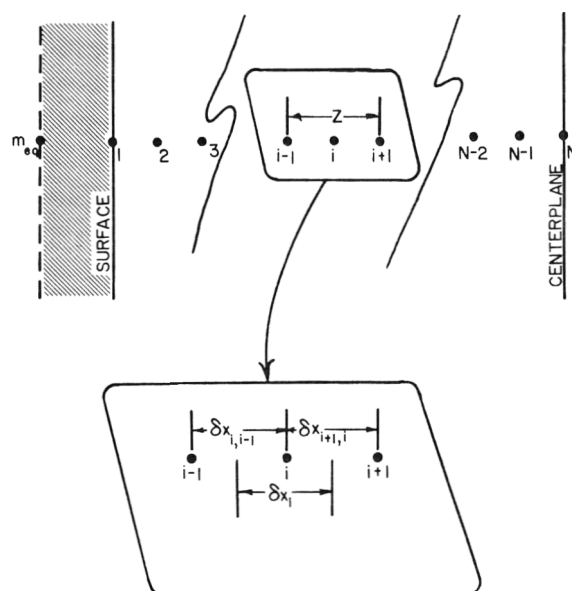
Fig. 4—Nodal format and notation used in solving the diffusion equation by a finite difference method.

portions of the probes were insulated to prevent heat conduction into the disk and therefore erroneous measurements. The detected temperature gradient was negligible. The surface area (A) and heat transfer coefficient (h) vary during drying due to shrinkage and other surface changes and have been estimated by equation 15:

$$hA = A_O (P'_1 M + P'_2) \qquad (15)$$

where $P'_1 = 0.0152$ and $P'_2 = 0.0237$ (hA is in cal/min°C). For simulation purposes m_s was approximated by Eq. 16:

$$m_s = \frac{\rho_O \pi d^2 L}{4(1+M_O)} \qquad (16)$$

where ρ_O is the initial density (approximately 1.04 g/cm³) of the potato disk with diameter (d) and thickness (L).

MAXIMIZING ASCORBIC ACID RETENTION

THE ABOVE MODELS may be used to simulate ascorbic acid degradation for specified drying conditions and provide a basis for selection of control schemes which improve ascorbic acid retention. The kinetic model developed for ascorbic acid kinetics was used in conjunction with the drying model to find optimal temperature control paths for maximizing ascorbic acid retention in potato disks. Numerical integration of the system of simultaneous differential equations was accomplished using Gear's method (Gear, 1971) to assure stability and increase numerical efficiency [DGEAR (IMSL) error tolerance 0.0001].

The complex method (Box, 1965) may be used to find the optimal temperature control scheme during drying using the methods described by Mishkin et al. (1982) and Mishkin (1983). For a batch process, discrete temperature decision points may be placed equidistant in time, between which a linear temperature profile is assumed. This was the first approach used to find drying regimes in this study. Numerical efficiency can be improved in the simulation if the temperature control path is not interrupted by the discontinuities inherent to such an approach. To eliminate these discontinuities, we investigated the use of a polynomial decision function and compared the results with the former method. Specifically, the dryer temperature was represented by the time-dependent polynomial:

$$T_{db} = a + bt_n + ct_n^2 + dt_n^3 + et_n^4 + ft_n^5 + gt_n^6 \qquad (17)$$

where $t_n = t/t_f$ and the parameters a, b, c, . . ., g are the decision variables, manipulated by the complex method in order to maximize the objective function,

$$\max J = C(t_f) \qquad (18)$$

where $C(t_f)$ is the final ascorbic acid content of the dried potato disks. A sixth order polynomial was used so that convergence times could be compared for the two approaches with the same number of decision variables, i.e. seven. A constraint was placed on the final average moisture content ($M_f \leqslant 0.05$ g/g-solids).

Reaction kinetics were evaluated as a function of the *average* moisture content of the drying potato disk in the initial phase of this study. This was the most appropriate approach since the models were derived on this basis (Mishkin, 1983). However, it is also extremely useful to study optimal control schemes taking into account the moisture distribution to find local ascorbic acid kinetics. By comparing optimization results using both local and average-moisture kinetics, we can determine the sensitivity of the methods. It is much simpler and numerically more efficient to evaluate kinetics on the basis of average moisture content than on a local moisture basis. Thus, if it were determined that insignificant differences were detected for the resultant optimal control schemes using both methods, the simpler approach would be followed.

Using local moisture kinetics for the optimization, the objective function was:

$$\max J = C_{avg} (t_f) \qquad (19)$$

where C_{avg} is the average ascorbic acid concentration within the disk. Ascorbic acid degradation rates were evaluated in each of the 10 volume elements. $C_{avg} (t_f)$ was calculated using Eq 20:

$$C_{avg} = \frac{(C_1 + C_{10})/2 + \sum\limits_{i=2}^{9} C_i}{9} \qquad (20)$$

where C_i represents the concentration of ascorbic acid in volume element "i". Diffusion of ascorbic acid within the potato tissue was ignored.

A FORTRAN version of the complex method by Evans (1975) was adopted for this work and that described below. In all cases the relative and absolute convergence criteria were 10^{-4} and 10^{-4}, respectively.

MINIMIZING DRYING TIME WITH A CONSTRAINT ON ASCORBIC ACID RETENTION

A MAJOR INDUSTRIAL CONCERN aside from product quality is processing time. By reducing drying time, for example, throughput can be increased along with productivity. A minimal-time control process was studied to show the utility of the complex method. Specifically, a nominal drying process was simulated at a constant temperature of 75°C for 190 min with a final moisture content of 0.05 g/g-solids. Using the ascorbic acid kinetic model, the final retention was determined for the simulated process (27%). This was used as a lower quality constraint in the minimum-time study as shown in Eq. 21:

$$c(t_f) \geqslant 0.27 \qquad (21)$$

The complex method was used with a time-dependent polynomial temperature decision function:

$$T_{db} = a + bt' + c(t')^2 + d(t')^3 + e(t')^4 + f(t')^5 + g(t')^6 \qquad (22)$$

where $t' = t/t^*$ and t^* is the length of the nominal drying process described above (190 min). Eq. 22 was also used in its linear form, i.e. with only parameters "a" and "b". The objective function for minimizing drying time is described by equation 23:

$$\min J = t_f \qquad (23)$$

For a given temperature control profile t_f is defined as the point in time during the numerical integration of the drying model where the moisture constraint is satisfied, i.e. where M = 0.05 g/g-solids.

OPTIMIZATION RESULTS

FIG. 1 SHOWS the moisture and temperature dependence of the first-order rate constant for degradation of ascorbic acid in white potatoes (Mishkin, 1983). The highest rate occurs in the moisture range 2 – 2.5 g/g-solids. Furthermore, the largest temperature sensitivity (highest activation energy) occurs in the high moisture range as shown in Fig. 2. Thus, one would expect that an optimal policy would be to keep the temperature low during the early phase of drying and then elevate the temperature as the moisture level drops.

Fig. 5 shows the optimal air temperature profiles for minimizing ascorbic acid loss using the polynomial decision function and the discrete decision approach for a drying process with length 180 min. Because ascorbic acid is stabilized when the product is dry the air humidity is maintained as low as possible to expedite drying and is not included as a decision variable. Similar results were obtained for drying processes of different durations. In general, these results are as expected. The small deviations which occur between the two approaches are not surprising.

By specifying the functionality of the temperature profile, e.g. a sixth order polynomial, we are imposing a constraint. It is probable that the "true" solution cannot be modelled exactly by this function. The discrete temperature decision approach similarly imposes a constraint on the possible profiles. Application of the maximum principle to drying optimization (Mishkin et al., 1982)

Table 2—Moisture transfer model parameter values for Eq. 7 and 8 (Mishkin, 1983)

Parameter	Value
P'_5	20.80
P'_6	85.34
P'_7	7.247
P'_8	23830.0
P'_9	45700.0
P'_{10}	7.006

does not impose such constraints on the time-dependent decision profile and therefore would be expected to find the "true" optimal path. This advantage, however, comes at great expense in view of the complications which accompany the method.

Taking into account these expected discrepancies, we are justified in concluding that the two decision approaches for the complex method yield essentially the same results. The temperature is maintained at lower levels during the early stages of drying followed by increased temperatures to satisfy the constraint on the final moisture content. As indicated in Fig. 5, ascorbic acid retention for the two appraoches is nearly identical. Therefore, although there are some differences between the optical control profiles generated, the resultant quality index is nearly identical. In a practical industrial application of the method this would indicate that the control profile used in an actual process should lie within the "range" of the indicated optimal paths, but need not track the profiles exactly.

As a basis for comparison of the optimization results with more conventional drying, a constant temperature process was simulated with equivalent final dryness, i.e. 0.05 g/g-solids. The simulated nominal process at 77°C resulted in 25% retention of ascorbic acid compared to 35% retention for the discrete-linear optimal temperature control path.

It is important to keep in mind that ascorbic acid stability is a function of the *sample* temperature and moisture content. Therefore, in Fig. 5 for the discrete-linear decision case, for example, although the air temperature is elevated during the early part of the drying process, the sample temperature remains much lower. This is illustrated in Fig. 6.

Fewer numerical problems were encountered using the polynomial decision approach. The discontinuous nature of the temperature control path using the discrete decision method caused some numerical simulation innaccuracies. In flat regions of the objective-function surface, these innaccuracies sometimes stalled the search. In order to insure that the discrete-linear temperature profile shown in Fig. 5 was actually optimal, several initial guesses were used to start the computer "runs." In addition, the convergence criteria were adjusted to assure that the search did not stop at a remote point. Using the polynomial decision, stalling was not experienced, and run times were generally shorter.
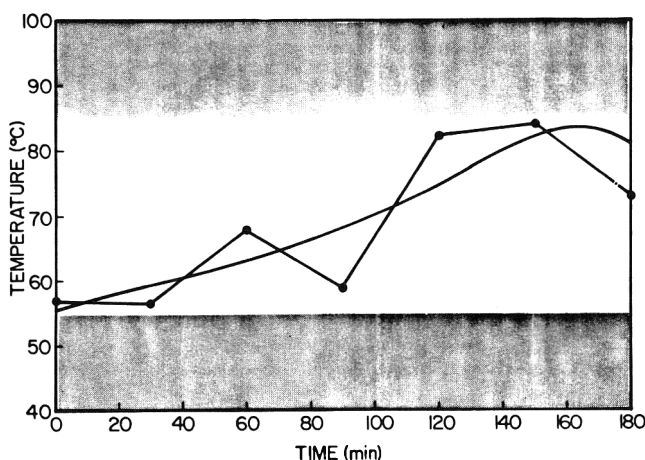
Optimal temperature control profiles are shown in Fig. 7 for kinetics based on the local and average moisture content, using the discrete decision approach of the complex method. There is excellent agreement between the optimal profiles. This is primarily because of the relation between the objective function and ascorbic acid kinetics. We have specified that the *average* retention of ascorbic acid is to be maximized at the end of the drying process, although a concentration gradient probably exists. Therefore, even in the case where the kinetics were evaluated while taking into account the moisture distribution in the disk, the index used to evaluate the process was based on the average ascorbic acid concentration.

Fig. 8 contrasts the nominal drying process at 70°C and the optimal processes for minimizing drying time. These are equivalent processes in terms of the final concentrations of ascorbic acid and moisture. In all cases the final ascorbic acid retention was 27%, i.e. the constraint on the final retention was active at the optimum as would be expected. The drying time was reduced from 190 to 127 min, using the 7 parameter form of the decision function (Eq. 22) and to 137 minutes, using the linear form. The temperature is lower during the early phase of the drying, becoming more elevated as the potato tissue becomes dryer and thus more stabilized toward ascorbic acid (see Fig. 1 and 2). The temperature must be elevated in order to reduce the drying time, yet lower while in the moisture sensitive region to satisfy the constraint on final retention of ascorbic acid. Such results are quite significant if, indeed, key quality factors can be modelled in industrial operations so that these techniques may be applied.

CONCLUSIONS

THE COMPLEX METHOD was proven to be a very reliable and simple approach for solving variational problems with state-variable inequality constraints. Application to air-drying optimization represents a rather severe test of the methods. The models used in this study are nonlinear and the resulting system of differential equations is stiff. Obviously, there are many more possible control variables other than air dry-bulb temperature which may be manipulated to improve drying processes. For example, bed depth or air flow rate might be more applicable in some instances. Application of the methods used in this study for such problems is straight-forward.

For optimization purposes there is always a trade-off between modelling efficiency and accuracy (Gill et al., 1981). Some work needs to be done to evaluate more completely the effects of modeling accuracy in drying with respect to optimization.

Fig. 5—Optimal dryer temperature control profiles using the polynomial and the discrete-linear decision functions for a 180 min process. The shaded area represents the constrained region. (Ascorbic acid retention: polynomial decision 34%, discrete decision 35%).
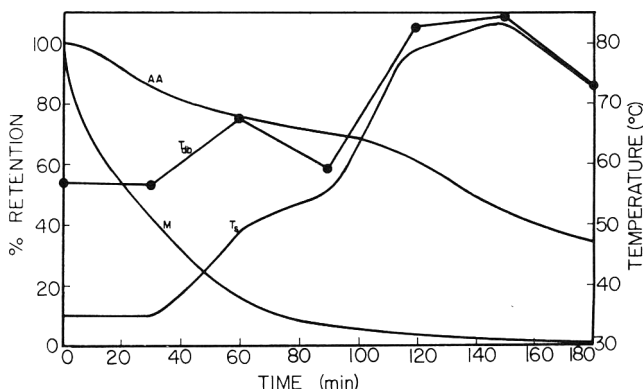
Fig. 6—Optimal dryer temperature (T_{db}) control profile, and the resultant sample temperature (T_s), moisture content (M) and ascorbic acid concentration (C) (180 min process).
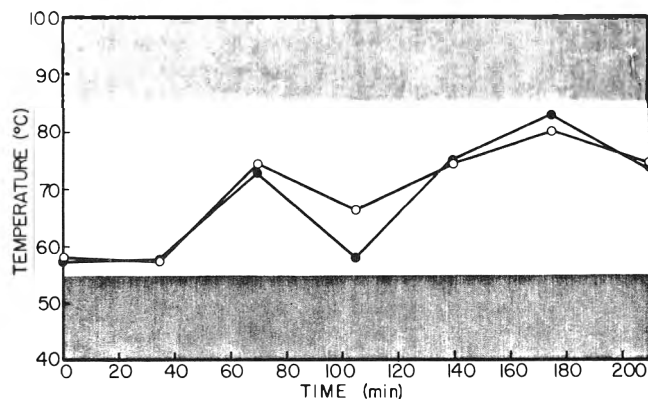
Fig. 7—Optimal dryer temperature control profiles using either average moisture content (−●−), or local moisture contents (−○−) in calculation of ascorbic acid retention (180 min process). The shaded area represents the constrained region.

Fig. 8—Optimal and nominal dryer temperature profiles for the final ascorbic acid retention of 27%, and a final moisture content of 0.05 g/g solids (PD − optimal using polynomial decision function; LD − optimal using the linear decision function; NP − nominal dryer profile).

SYMBOLS

A	Area, (cm^2)
A$_o$	Initial area of sample, (cm^2)
a-g	Parameters in Eq. 17 and 22
C	Concentration of ascorbic acid (normalized with respect to initial concentration)
C(t)	C as function of time
D	Diffusion coefficient for water, (cm^2/sec)
D$_N$	D in centerplane node (Fig. 4)
D$_{ref}$	Reference D, (cm^2/sec)
d	Sample diameter, (cm)
E$_a$	Activation energy for destruction of ascorbic acid, (cal/mole)
E$_D$	Activation energy for diffusion , (cal/mole)
J	Objective function
h	Heat transfer coefficient, [cal/(min$^\circ$C)]
k	Reaction rate constant, (min^{-1})
k$_o$	Constant, (min^{-1})
L	Sample thickness, (cm)
M	Moisture content, (g/g solids)
M$_{eq}$	M in equilibrium with the atmosphere
M̄	Average M
M$_N$	M in centerplane node (Fig. 4)
M$_o$	Initial M
m$_s$	Weight of solids in sample
N	Number of volume elements (Fig. 4)
P$_1$−P$_7$	Parameters in Eq. 3 and 4
P$'_1$−P$'_2$	Parameters in Eq. 15
P$'_3$−P$'_4$	Parameters in Eq. 12
P$'_5$−P$'_{10}$	Parameters in Eq. 7 and 8
R	Gas constant, (cal/mole $^\circ$K)
T	Temperature, ($^\circ$K)
T$_{db}$	Dry bulb temperature, ($^\circ$C)
T$_s$	Sample temperature, ($^\circ$C)
T$_{ref}$	Reference temperature, ($^\circ$K)
t	Time.
t*	t for nominal drying process (min)
t'	Normalized time, Eq. 22
t$_f$	Drying time, (min)
t$_n$	Normalized time, Eq. 17
x	Distance in the sample, (cm)
δ_x	Distance between nodes (Fig. 4) (cm)
λ_w	Latent heat of vaporization (cal/g)
ρ_o	Initial density of sample (g/cm^3)

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A Dynamic Test for Kinetic Models of Chemical Changes During Processing: Ascorbic Acid Degradation in Dehydration of Potatoes

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─────────ABSTRACT─────────

A method was developed for kinetic-model parameter estimation using data obtained from processes with known dynamic stress paths. The utility of the method was demonstrated for the case of modeling of ascorbic acid degradation during air drying of potatoes. Potato discs were dried under a variety of conditions using a laboratory-scale tray-dryer with wet- and dry-bulb temperature control. Ascorbic acid concentration was monitored as a function of time along with average moisture content and temperature. These data obtained under dynamic conditions were used to estimate optimal parameters in a kinetic model describing the rate of degradation of ascorbic acid as a function of temperature, moisture content, time, and ascorbic acid concentration.

INTRODUCTION

MATHEMATICAL MODELS of chemical changes occurring in processing of foods, drugs and other sensitive material are of substantial value to process development. Such kinetic models are essential to development of optimization procedures for such processes (Evans, 1982). The development of kinetic models for degradation of food and drug quality during thermal processes has received increasing attention in recent years, and has been aided by increasing availability of appropriate computer software (Saguy and Karel, 1980; Labropoulos et al., 1981; Ohlsson, 1980; Horak and Kessler, 1982; Mollica et al., 1978; Lund, 1982).

Dehydration is a process of particular interest in a number of applications in biotechnology since it is often the method of choice for stabilization of foods and pharmaceuticals. An example of chemical changes of relevance in dehydration is the degradation of vitamins and of other nutrients in foods. Many nutrients undergo deterioration during dehydration (Karel, 1979; Villota, 1978; Labuza, 1972, 1973). In addition, many other quality attributes are affected, such as the degree of browning which is intimately connected with nutrient losses (Hendel et al., 1955; Mauron, 1981). An obstacle in the path of maximizing nutrient retention during drying is the lack of adequate kinetic models (Lund, 1983; Luyben, 1980; Karel, 1979; Labuza, 1972). In general, the degradation kinetics of nutrients are a function of moisture and temperature. Unfortunately, a food product traverses a large range of temperature and moisture during drying. Thus, a kinetic model must necessarily be representative over this range. Usually, such modeling is done using data from static experiments using a balanced matrix design (Mizrahi and Karel, 1977a, b). In such a design, if, for example it was determined that reaction kinetics were first order, one would obtain a matrix of first-order rate constants at various levels of moisture and temperature. The moisture and temperature functionality of the rate constant could be determined

from such data. It is obvious that many experiments are required in such a scheme.

The possibility of deriving kinetic model parameters, or predictions of extents of chemical changes from dynamic experiments in which the factors affecting the rates of reaction (e.g. temperature and moisture) are changing in a known manner, has been considered by several investigators. Nonisothermal accelerated stability tests have been used by a number of workers (Zoglio et al., 1968; Tucker, 1981). Mizrahi and Karel (1977a, b, 1978) used a dynamic method involving programming the rate of moisture gain by a dehydrated food, to predict extent of nutrient degradation during storage.

Saguy et al. (1978) proposed a dynamic method for determination of kinetic models of nutrient loss and of other chemical changes from data obtained during dehydration. The dynamic method for determining nutrient degradation kinetics, under conditions representative of drying, requires the acquisition of moisture, temperature, and nutrient concentration data from representative samples during actual drying experiments, i.e. under nonstatic conditions. These data are used to determine numerically the best set of parameters for a proposed kinetic model.

We investigated the use of the method in the development of models for degradation of vitamin C in potatoes. The selection of ascorbic acid was not arbitrary. Its stability is very sensitive to processing conditions and is quite indicative of product quality.

MATERIALS & METHODS

Variables monitored

Air drying experiments were conducted in which the "state variables" and the "control variables" were monitored as a function of time (t) during the drying. The state variables included sample temperature (T), the average moisture content (M), and the average concentration of ascorbic acid (C). The monitored control variables were the air dry and wet bulb temperatures (T_{DB} and T_{WB}). The control history is not needed for developing the kinetic model, but is required to construct the moisture and temperature models needed for simulation of drying (Mishkin, 1983).

Data collection

Whole unpeeled white potatoes were sliced perpendicularly to the long axis into slabs, 4.0 mm thick with a precision meat slicer and cored with a sharpened stainless steel pipe to form thin disks 4.1 cm diameter. Individual sample thicknesses were maintained within a 5% deviation. The slices were placed in a centrifugal fan shelf dryer with dry bulb (T_{DB}) and wet bulb temperature (T_{WB}) controls. The dryer was modified so that sample trays revolved in a Ferris wheel-like device. This modification assured that each sample tray was exposed to the same air flow pattern resulting in uniform drying. Thermocouple probes (sheathed in 0.75 mm thick hypodermic needles) were inserted radially into the center of assorted samples. Sample trays had screen bottoms so that drying occurred from both sides of the potato disks. Thermocouple probes were connected to a data logger which recorded T, T_{DB}, and T_{WB} at 1 min intervals. The uniformity of sample temperature had been demonstrated previously (Mishkin, 1983). At selected times two sample trays (30 samples) were withdrawn from the dryer at random. The samples were weighed, within 30 sec of oven withdrawal, and the diameter and thickness of the disks measured. The samples

were then cooled rapidly in liquid nitrogen to prevent further degradation of ascrobic acid. Each sample was freeze-dried for 48 hr at 23°C. Special care was required to prevent thawing after freezing and before freeze drying in order to prevent residual ascorbic acid oxidase from acting on ascorbic acid. It was demonstrated that freezing and thawing destroyed the integrity of membranes which serve as compartmentation barriers in the native state, isolating the enzyme from the substrate.

After freeze drying, the samples were weighed immediately to determine the total solids. The moisture contents at the sampling times were then determined by difference. The freeze-dried samples were ground to a fine powder in a blender and stored under dry nitrogen at −25°C in darkness for up to 48 hr until ascorbic acid was assayed using a high pressure liquid chromatography (HPLC) method. One gram of sample powder was accurately weighed into a 50 mL centrifuge tube and extracted with 25 mL 8% acetic acid for 1.5 hr at room temperature in darkness, then centrifuged and refrigerated before HPLC injection. The extract was filtered (pore size 0.45 μm). Two mL of the filtrate were diluted to 10 mL using the mobile phase solution immediately prior to HPLC injection (0.25% citric acid:methanol, 40:60 v/v). A stainless-steel (25 cm × 4.6 mm i.d.) S5 NH2 column (5 μm particle size) was used with a Waters model 6000A solvent delivery system. Peak area was determined using a Hewlett-Packard model 3390A integrator. Ascorbic acid was detected at 254 nm. Standard deviation for the assay was 3.5%.

Determination of kinetic model parameters

The basic scheme used for the dynamic test is outlined in the flowchart of Fig. 1. Five drying experiments were run using different programs for changing T_{DB} with time (Mishkin, 1983) so that a broad data base would be available for the subsequent modeling. For a proposed kinetic model with temperature and moisture functionality, each experiment could be simulated using the *known* temperature and moisture history. Such a simulation can be performed for all (or any) of the experiments simultaneously for a particular set of parameters. During the simulation, for points in time intermediate to data points, moisture and temperature may be approximated by interpolation. Semilog-linear interpolation was used for moisture.

$$M(t) = M_i + \exp\left[(t-t_i)\frac{\ln(M_{i+1}/M_i)}{t_{i+1} - t_i}\right] \quad (1)$$

For the temperature data linear interpolation was considered most appropriate. Using a multivariable minimization routine, the best set of parameters may be estimated by minimizing an objective function such as Eq. 2:

$$\min J = \sum_{i=1}^{n} w_i \sum_{j=1}^{m} \left[\frac{C_{obs_{ij}} - C_{calc_{ij}}}{\sigma_{ij}}\right]^2 \quad (2)$$

$C_{calc_{ij}}$ is the *simulated value* for concentration for the j-th observation of the i-th experiment. $C_{obs_{ij}}$ is the j-th observation for the i-th drying experiment, and n and m are the number of experiments and the number of observations per experiment, respectively. W_i is a weighting factor for data set "i" and σ_{ij} is the standard deviation of $C_{obs_{ij}}$. C(t=0) is left as an independent variable (for each experiment) to be found by the minimization routine. Inclusion of C(t=0) as an independent variable reduces the fit bias which would result if C(t=0) were specified. A direct search method, the complex method, has proven to be very reliable in this study for parameter estimation. A quasi-Newton method (ZXMIN; IMSL Library) has also been used in some cases.

There are no standardized techniques for parameter estimation using models which are expressed by differential equations, evaluated under *dynamic conditions*. Goodness of fit can only be determined by careful examination of the final residuals and by visual inspection of the fits.
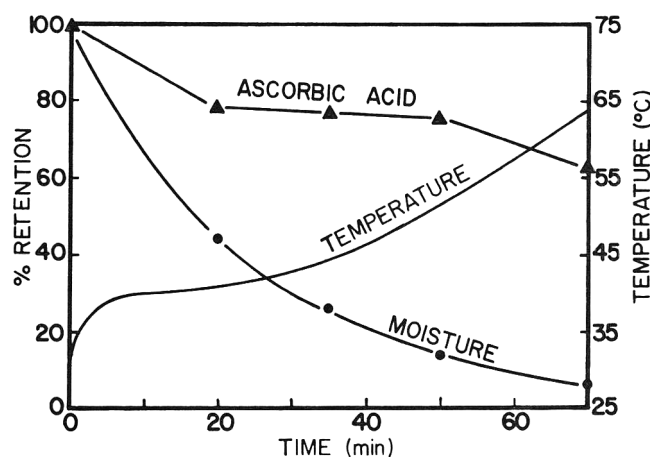
Fig. 2—Time profiles for moisture and ascorbic acid retention, and temperature for one of the five drying experiments.

Fig. 1—Schematic representation of procedure for parameter estimation using dynamic tests.
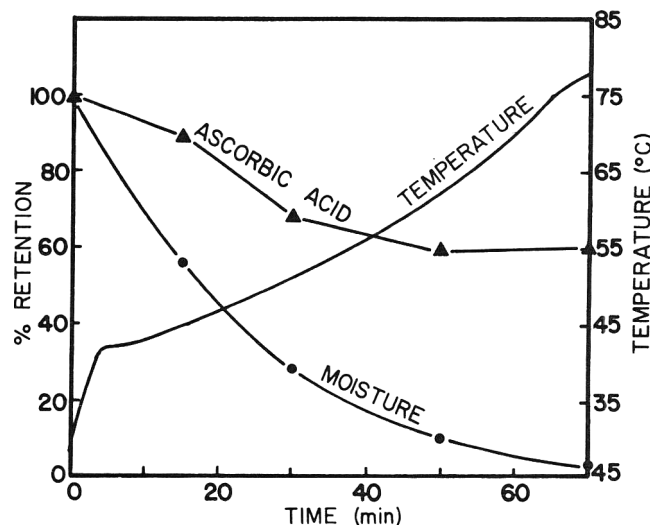
Fig. 3—Time profiles for moisture and ascorbic acid retention, and temperature for one of the five drying experiments.

RESULTS & DISCUSSION

FIG. 2 AND 3 SHOW the data for two of the five separate drying experiments. Typical initial concentrations were 0.27 mg/g-solids (range 0.24–0.29) for ascorbic acid and 3.7 g/g-solids for water in the potato tissue. The method used was described in the previous paper. The experiments were run under widely different conditions so that a broad data base would be generated.

There are specific problems associated with using a dynamic test for determining the kinetics of a reaction, especially when two variables are varied simultaneously, i.e. moisture and temperature. When using the classical balanced matrix data acquisition method (static test), one has data

Table 1—Ascorbic acid kinetic model parameter values for Eq. 3 and 4 (Mishkin, 1983)

Parameter	Value
P_1	16.38
P_2	1.782
P_3	1.890
P_4	14831.0
P_5	241.1
P_6	656.2
P_7	236.8
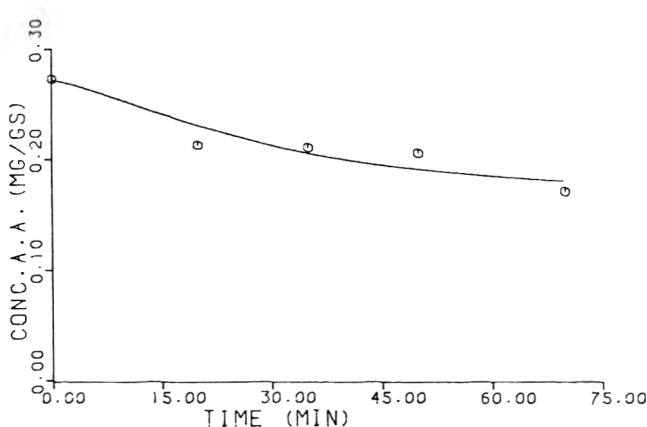
CONCENTRATION VS. TIME

Fig. 4—Ascorbic acid retention calculated from simulation based on the kinetic model, compared with experimental results.
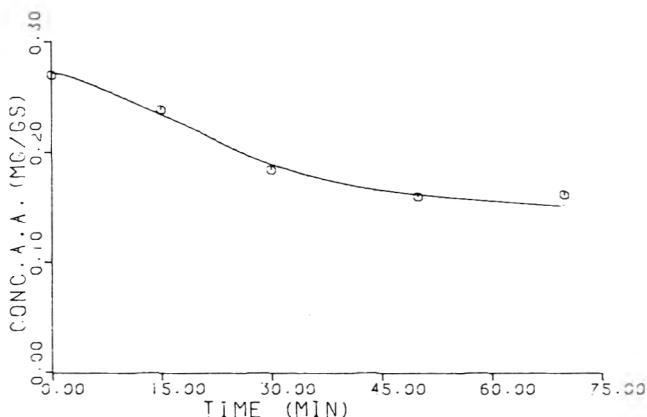
CONCENTRATION VS. TIME

Fig. 5—Ascorbic acid retention calculated from simulation based on the kinetic model, compared with experimental results.

in a form which renders intuitive model selection possible on the basis of visual inspection of the data. Using the data obtained by the dynamic method one may be able to ascertain qualitative aspects of the specific sensitivities of the nutrient to factors such as moisture, but there is no way to make a reasonable guess for the form of a representative model. Thus, for such an approach to be useful one must have available a "good guess" for the form of the model from theory or the literature, or use a model of sufficient flexibility to assure an adequate fit. First-order kinetics were tested for modeling ascorbic acid kinetics,

$$-dC/dt = kC \qquad (3)$$

where C is the concentration of ascorbic acid (normalized with respect to the initial concentration).

Arrhenius temperature dependence was used to model the first-order rate constant,

$$k = k_o \exp[-E_a/RT] \qquad (4)$$

where k_o and E_a were functions of moisture content,

$$\ln k_o = P_1 + P_2 M + P_3 M^2 \qquad (5)$$

$$E_a = P_4 + P_5 M + P_6 M^2 + P_7 M^3 \qquad (6)$$

M is the moisture content in g/g-solids, and P_1–P_7 are parametric constants to be estimated using the proposed modeling method.

Simulated results for two of the five data sets for the estimated parameters (Table 1) are shown in Fig. 4 and 5 along with the data for the experiments. The moisture and temperature dependence associated with this model is shown in Fig. 6. The moisture dependence of the activation energy is illustrated in Fig. 7 and the residual plot for the
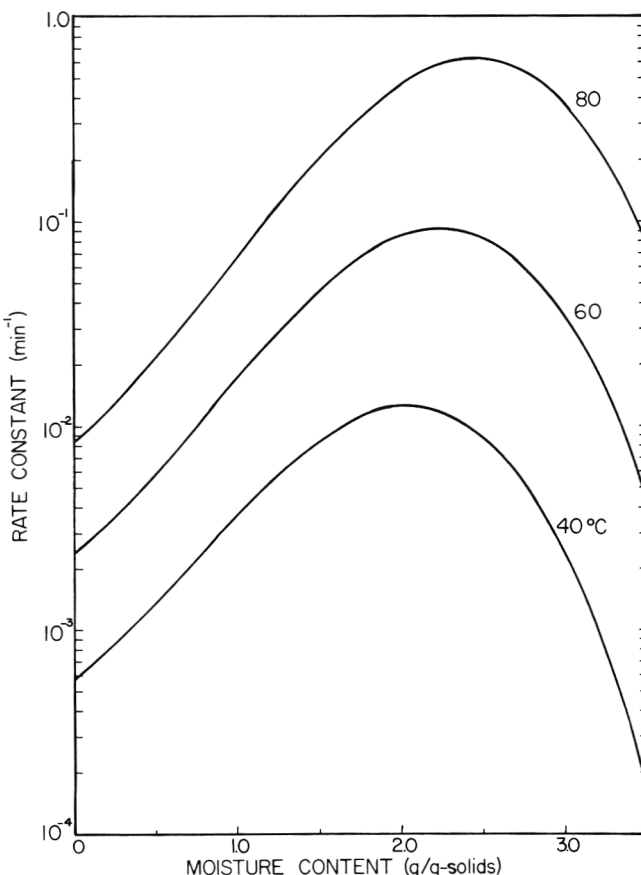
Fig. 6—Temperature and moisture dependence of the first order rate constant for ascorbic acid destruction, as given by the kinetic model.
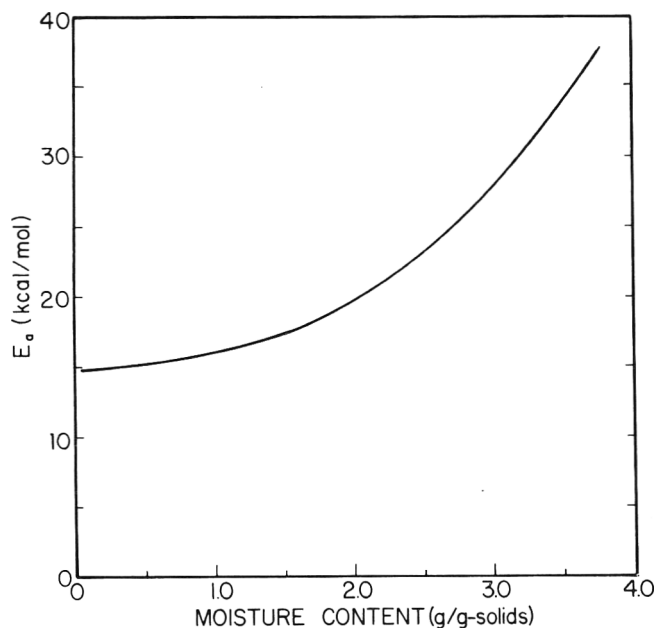
Fig. 7—Dependence of activation energy on moisture content.

Fig. 8—Residual plot for fit of the kinetic model to all experimental results. Residuals are given as fractions of the measurement and regression standard errors.

combined fit is shown in Fig. 8. Efforts were made to reduce the number of terms in the model, but without success. Different polynomial models for moisture and temperature dependence were also tried (Mishkin, 1983) but the number of parameters was not reduced. Use of simpler or more appropriate models using the dynamic method is very difficult in view of the dynamics of the data acquisition. It should be noted that it was important that the models presented here be sufficiently accurate to warrant their use in optimization, yet easily and efficiently evaluated (Gill et al., 1981). As a word of caution, models containing large numbers of parameters can lead to multimodal functions within the range of the data, especially for sparse data sets and polynomial functionalities. For the models presented here this was not the case.

There is a rapid decrease in the rate constant at high moisture levels (Fig. 6), due to physical circumstances under which the kinetic data were obtained. During the early stages of drying, the membrane integrity of the potato tissue was substantially intact, protecting ascorbic acid from deleterious cell components. As drying proceeded and the moisture level dropped (and the temperature increased) this compartmentation may have been lost resulting in accelerated kinetics. It is also possible that endogenous antioxidative constituents may be responsible for this initial low rate. In either case, the dynamic method views the phenomena as a diminished rate at high moisture levels and adjusts the parameters accordingly. It is unlikely that a static test would reveal this, and thus would be less effective in modeling actual losses during drying.

The kinetic model developed in this study and, in particular, the estimated parameters are representative of the particular batch of potatoes used. Because of the complexity of plant tissue one might expect different varieties of potatoes or potatoes of identical varieties, but different preprocessing histories, to have somewhat different ascorbic acid degradation kinetics. Before processing, potato tissue maintains metabolic activity which continues to change the chemical environment of the tissue. For example, storage temperatures can alter the concentration of reducing sugars. Also, note that the potatoes in this study were not blanched. For industrial practice, it might be important to find models which are generally effective for a broad spectrum of raw materials. Whatever the requirements, it will be necessary to assure that the data base is representative of the ultimate goal
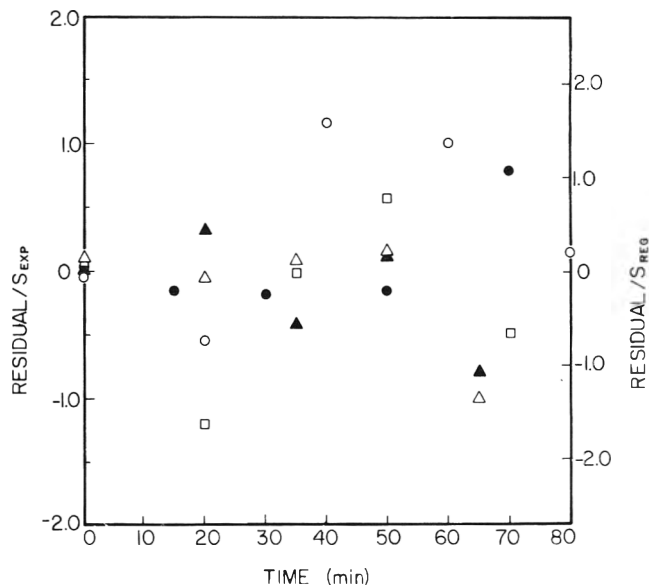
The model parameters were derived using the average moisture content of potato disks of a specific geometry, thus ignoring local moisture effects. Such a model, although very effective for a particular case, might be less representative for other shapes and sizes. Due to the moisture gradient, one would expect a gradient in the rate of destruction of ascorbic acid (or any other nutrient) as well. For industrial applications of the dynamic method it will be important to keep in mind the possible effects of moisture gradients due to the transient nature of the approach, when designing experiments which should be representative of a particular process.

SYMBOLS

C	Concentration of ascorbic acid (normalized with respect to initial concentration)
C_{obs}	Observed C
C_{calc}	C calculated using kinetic model
E_a	Activation energy, cal/mole
J	Objective function defined in Eq. 2
k	Reaction rate constant, min^{-1}
k_o	Constant, min^{-1}
M	Average moisture content, g/g solids
$M(t)$	M as a function of time
$P_1, P_2, P_3, P_4, P_5, P_6, P_7$	Parameters in Eq. 5 and 6
R	Gas constant, (cal/mole °K)
t	Time, min
T	Sample temperature, °K
T_{DB}	Dry bulb temperature of air, °C
T_{WB}	Wet bulb temperature of air, °C
w_i	Weighting factor defined in Eq.2
σ	Standard deviation mg/g solids

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Effect of Xanthan Gum Upon the Rheology and Stability of Oil-Water Emulsions

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────────ABSTRACT────────

Soyabean oil-water emulsions were studied. In oil-water emulsions (up to 60% oil) xanthan is essential to prevent creaming. A yield stress arises primarily from the polysaccharide liquid crystalline structure. In concentrated systems where the oil droplets interact strongly there is a significant contribution to the yield stress arising from the need to modify individual droplet shapes when shear is applied to the system. Studies of droplet sizes suggest that the xanthan gum can also modify the equilibrium droplet size by lowering the oil-water interfacial tension.

INTRODUCTION

OIL-WATER EMULSIONS occur in many industrial processes and are the basis of many products. In the food industry such products are typified by salad creams and mayonnaise where soya oil is widely used having generally replaced olive oil which is more expensive. The main rheological characteristic of salad cream and mayonnaise is the presence of a yield stress. Creep recovery behavior is displayed by several food systems. When a system exhibits yield behavior, a finite amount of stress is needed for the initiation of flow. This is of particular advantage in storage of the food emulsions, where very low values of stresses are involved, usually lower than that necessary for the onset of flow. As a result, the possibility of any structural changes taking place during storage is minimized. On the other hand, a yield stress can sometimes be undesirable. Golden syrup is considerably more viscous than tomato ketchup. However, it is easier to pour than the ketchup. This can be attributed to the fact that ketchup displays yield behavior, while syrup does not (i.e. it starts flowing as soon as the stress is applied).

Another important parameter is emulsion stability, with respect to creaming and coalescence. In order to enhance the stability, various polysaccharide stabilizers such as xanthan gum, or alginates are used in the preparation of mayonnaise and salad creams. Several investigators have studied the rheology and stability of oil-water emulsions. Vernon-Carter and Sherman (1981) have investigated the effect of different amounts of mesquite gum and Tween emulsifiers on rheology and stability of corn oil-water emulsions. The properties of these emulsions were found to depend upon relative amounts of the two emulslsifiers present in the emulsions. Emulsifying behavior of various proteins has been studied by Tornberg (1980). Tornberg and Herman (1977) have investigated the effect of processing on oil-water emulsions by using several techniques for preparation of emulsions. Rheological studies of commercial mayonnaise and salad creams has been carried out by Elliott and Ganz (1977). However, a systematic study designed to identify the role of stabilizers in emulsions similar to mayonnaise and salad creams seems to be lacking. In this paper, we study the effect of xanthan gum upon the rheology and stability of soya oil-water emulsions, by comparing the properties of emulsions with and without xanthan gum. Egg

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yolk was used as an emulsifier since it is used commercially in mayonnaise and salad creams.

MATERIALS & METHODS

Water

Deionized water was used for the preparation of emulsions. Deionization was carried out in two stages. The first stage was a TSA 300 twin bed with weak base anion resin. The second stage was a MB 12 mixed bed. Both the twin bed and the mixed bed were supplied by Permutit Services Ltd.

Soyabean oil

A commercially available soyabean oil, supplied by Petty, Wood & Co. was used. The fatty acid composition was 16:0, 9.8%; 18:0, 3.9%; 18:1, 20.1%; 18:2, 51.0%; 18:3, 7.1%.

Egg yolk

Dried egg yolk powder was supplied by Colman's of Norwich. Xantahn gum was ex KELCO/AIL and was supplied by Colman's of Norwich.

Preparation of emulsions

Two series of emulsions were prepared. Series A was prepared using xanthan gum as stabilizer, while series B was prepared without xanthan gum. Emulsions were prepared using a Waring Commercial food blender. The emulsions were sheared typically for 2–3 min. Table 1 lists the compositions of emulsions.

Particle size distribution measurement

Several methods have been used in the past for particle size distribution measurement. These include photon correlation spectroscopy (Cummins and Pike, 1974), light scattering (Kerker, 1969), Coulter Counter (Wachtel and LaMer, 1962) and turbidometry (Doty and Steiner, 1950). Most of them involve diluting the emulsion by a considerable amount. In the present work, a Malvern particle size analyzer model 2600 MSD was used. This instrument uses the principle of Fraunhofer diffraction where a parallel, monochromatic beam of laser (red light $\lambda = 633$ nm) illuminates the emulsion. The light diffracted by the emulsion droplets gives a stationery diffraction pattern regardless of the particle movement. As particles enter and leave the illuminated area, the diffraction pattern changes, always reflecting the instantaneous size distribution in the illuminated area. A Fourier transform lens focuses the diffraction pattern onto a multi-element photoelectric detector which produces an analog signal proportional to the received light energy. The detector is interfaced to a desk top computer for data analysis. As with other

Table 1—Composition of soya oil-water emulsions

Emulsion no.	Soybean oil (Wt %)	Water (Wt %)	Egg yolk powder (Wt %)	Xanthan (Wt %)
A1	20	76	3	1
A2	40	56	3	1
A3	60	36	3	1
A4	70	26	3	1
B1	20	77	3	—
B2	40	57	3	—
B3	60	37	3	—
B4	70	27	3	—

methods, it was necessary to dilute the emulsions, the volume fraction of oil in the diluted emulsion being less than 10^{-3} in all cases. The emulsions were diluted with deionized and distilled water, and no dispersing agent was necessary for dilution. The Malvern instrument then offers a rapid, easy and reproducible method of particle size measurement.

Rheology. Measurement of viscosity as a function of shear rate were carried out using a Contraves rheometer model 115. This intrument is a rotational viscometer, using a concentric cylinder assembly. The outer cylinder is stationary while the inner cylinder can be rotated at various speeds. The shear rate is a function of rotational speed, while the shear stress is proportional to the breaking torque. The measurements were carried out at 22°C. The particle size analysis and rheological measurements were performed within 24 hr of the preparation of emulsions.

Creaming. Measured amounts of emulsions were poured into measuring cylinders of identical size and stored in a refrigerator. The height of the clear liquid at the bottom was measured at the end of 24 hr (Table 2). A series emulsions did not exhibit any creaming behavior.

RESULTS & DISCUSSION

EMULSION STABILITY was observed with respect to creaming and coalescence. Coalescence is indicative of emulsion instability, where two or more droplets coagulate to form larger droplets. The droplets thus coalesce into progressively larger masses of oil, which in extreme cases may result in a continuous oil phase separated from water by a single interface. All the emulsions were stable with regard to coalescence over a period of 4 wk, in that no large droplets of oil were observed at the top. It is quite possible, however, that coalescence may have occurred on a microscopic scale. The observations were distontinued after 4 wk. Onset of fungoid growth could be observed approximately 6 wk after the preparation of the emulsions and hence, observations were considered to be unreliable 2 wk prior to that.

In creaming, the emulsion droplets separate from the continuious phase, flocculate and move towards the top or the bottom, depending upon the density difference between the continuous and disperse phases (Vold and Vold, 1983). Creaming by itself is not indicative of emulsion breakdown. However, creaming can facilitate coalescence, which results in emulsion breakdown. There is an important difference between creaming and coalescence: creaming is reversible while coalescence is irreversible. In the creaming process, the emulsion droplets separate from the continuous phase, while coalescence involves two or more droplets coming together and forming a larger droplet, thereby destroying the original emulsion in the process. It is well known that food emulsifiers (proteins, gums or lipids) are able to produce emulsions of almost unlimited persistence with regards to coalescence (Dickinson and Stainsby, 1982). Hence, no attempt was made to study coalescence in this work, and emulsion stability was studied only with respect to creaming.

The 20% emulsion was most susceptible to creaming, while 60% emulsion (B3) was the least susceptible (Table 2). The B4 emulsion was stable for 4 wk without creaming and was comparable to the A series emulsions, rather than the other members of the B series. During the creaming process the oil droplets tend to migrate towards the top, because of the density difference. In 20% emulsion, there were relatively fewer droplets present and hence an individual droplet encountered little resistance towards upward migration. However, in the emulsion B4, the oil droplets were packed very closely together (because of high concentration of oil); therefore, there was a great deal of resistance encountered by a droplet during upward migration, resulting in the absence of creaming. B2 and B3 fall in between the two extreme cases, with B2 creaming more easily than B3. The phenomenon observed here was creaming, and not syneresis (since no thickener was used in the preparation of B series emulsions).

Fig. 1 and 2 show the plots of viscosity vs shear rate for the A series and B series emulsions, respectively. Both series of emulsions exhibited typical shear thinning behavior, indicative of the breakdown of emulsion aggregates at high shear rates. Emulsions B1 and B2 were almost Newtonian in character, with a limited amount of shear thinning. B3 exhibited viscoelastic behavior to some degree, while B4 was strongly visco-elastic. B4 also exhibited a yield stress, while no yield stress was observed for the other B emulsions. Clearly, there was a trend towards increased visco-elasticity with increasing oil content. However, B4 behaved differently from the other B emulsions in that it was considerably more viscous and had a yield value. Thus, the anomalous behavior of the B4 emulsion was confirmed by rheological measurements.

In a dilute emulsion such as B1, the droplets may be assumed to be spherical, since this minimizes the surface area and hence the droplet free energy. With increasing oil content, the droplets are packed more and more closely. If the droplets are assumed to be rigid and nondeformable (e.g. as in polystyrene latex), there is an upper limit of 64% for the random close packing of uniform spheres in three

Table 2—Creaming behavior of soya oil-water emulsions

Emulsion no.	Ht of clear liquid column at end of 24 hr (m)
B1	0.010
B2	0.004
B3	0.002
B4	0
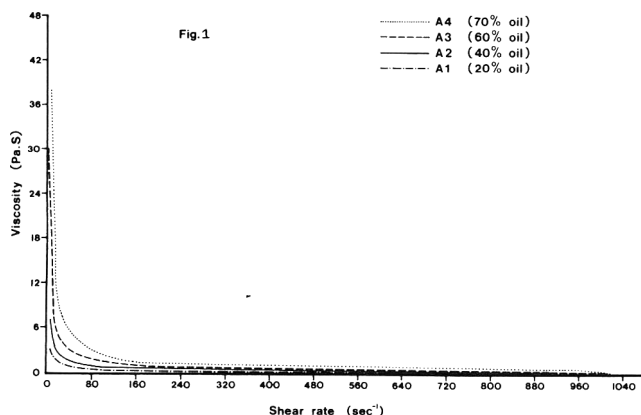
Fig. 1—Viscosity vs shear rate plots for A series of emulsions.
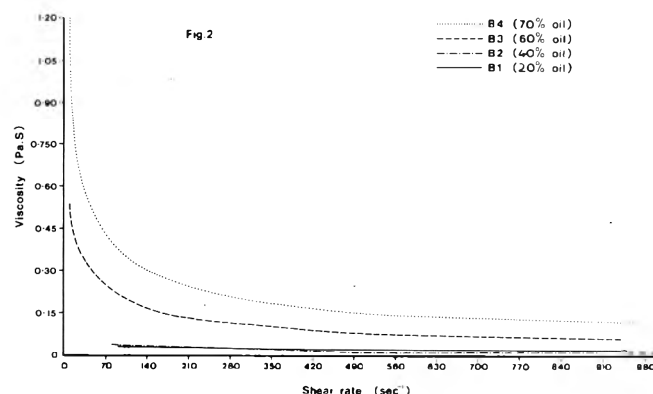
Fig. 2—Viscosity vs shear rate plots for B series of emulsions.

dimensions (Scott, 1960). For a polydisperse emulsion, this figure would be greater, since the smaller droplets can be packed in the void spaces formed by the packing of larger droplets. For the B4 emulsion, the volume fraction of oil is in this dense close packed regime and there will be a considerable amount of droplet-droplet interaction. Molecules of egg yolk proteins, which act as emulsifier, are adsorbed at the oil-water interface. When the droplets are close together or almost touching each other, strong interactions between protein molecules can be expected. These interparticle interactions were probably responsible for the enhanced stability of B4 emulsion.

When shear was applied to such a concentrated emulsion, it caused the droplets to move and slip relative to each other. The droplets continuously undergo deformation and relaxation of the spherical shape, resulting in storage and dissipation of energy. This storage of energy (which resembles the action of a coiled spring), coupled with strong interparticle interactions, gives rise to the yield behavior and visco-elastic behavior of the emulsion B4.

All the A series emulsions were visco-elastic (Fig. 1) and exhibited yield behavior (Table 3). The differences in rheological and creaming behavior of the two series of emulsions could be attributed to xanthan gum. It has been well established that xanthan gum forms a weak microgel type structure in water (Morris, 1977; Maret et al., 1981; Milas and Rinaudo, 1981). Since water was the continuous phase in the emulsions studied, we can assume that xanthan forms a microgel in the A series emulsions, which keeps the emulsion droplets physically suspended in the medium, thereby preventing the creaming process. The stability towards creaming was due to xanthan gum for the A series emulsions, while for the B4 emulsion, it was due to strong interparticle interactions.

The liquid crystalline structure of xanthan gum microgel probably also accounted for the rheological behavior of The A series samples. 20%, 40% and 60% emulsions exhibited very little visco-elasticity without xanthan gum, while they were strongly visco-elastic with xanthan gum and

exhibited yield stress. In the case of 70% oil, the emulsion without xanthan gum (B4) was visco-elastic, while the emulsion with xanthan gum (A4) was much more viscous and had a much greater yield value. Thus, the major contribution to yield behavior came from xanthan gum. It is quite likely, however, that the emulsion droplets reinforced the gel structure, acting as fillers. We are currently studying this aspect in greater detail.

The particle size of the mayonnaise sample was comparable to that of the A series emulsions (Table 4), and in agreement with previously reported results (Tung and Jones, 1981). The particle size for the A series samples was not influenced by the amount of oil in the emulsions. Fig. 3 shows the typical droplet size distribution for the two series of emulsions. B series emulsions were much more polydisperse than the A series. Another striking feature is that the average particle size of B series emulsions is an order of magnitude greater than that for the A series. Both the A and B series emulsions were prepared under identical conditions involving comparable shear rates. In the A series, when the droplets were formed, they were kept apart by the highly viscous xanthan gum microgel and prevented from coalescing into larger droplets. In the B series, the collisions between individual droplets were more

Table 3—Yield stress values for soya oil-water emulsions

Emulsion no.	Yield value, N/M
A1	13
A2	32
A3	71
A4	104
B1	—
B2	—
B3	—
B4	7

Table 4—Average (median) particle sizes for soya oil-water emulsions

Emulsion no.	Average particle size, μm	Standard deviation
A1	2.4	2.6
A2	3.0	3.2
A3	2.8	3.0
A4	2.9	3.0
Mayonnaise (70% oil-water)	2.6	
B1	20	25
B2	22	28
B3	13	16
B4	11	12
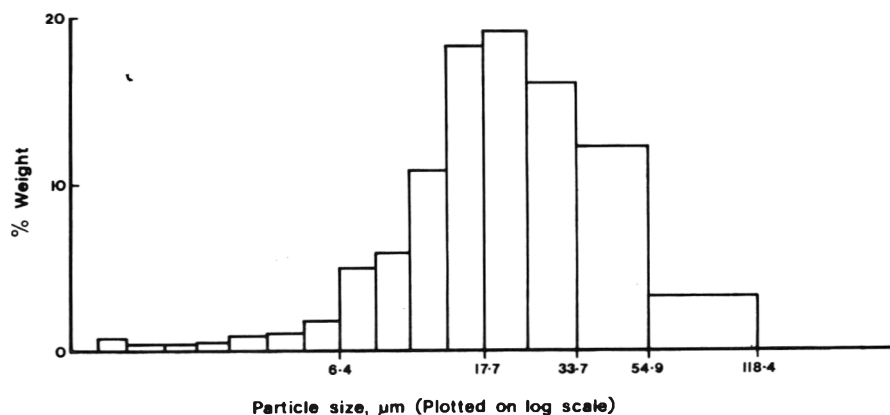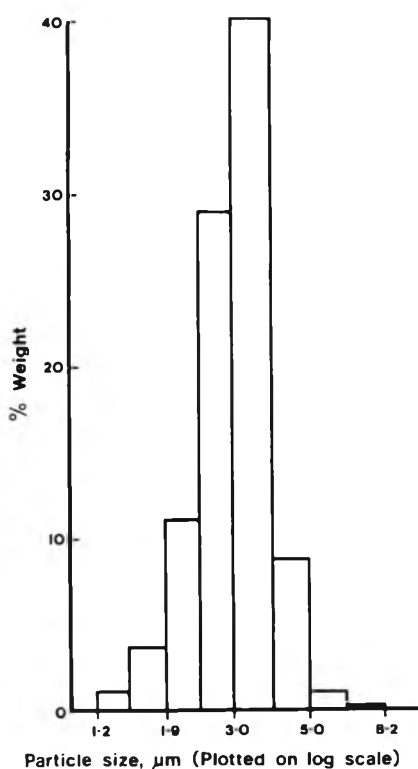
Fig. 3—Particle size distributions for (a) A2 and (b) B2 emulsions.

frequent (because of the low viscosity of the emulsions) and hence, it may be easier to form larger droplets. Smaller droplets tend to be more stable towards coalescence than larger droplets (Dickinson and Stainsby, 1982). The large droplets tend to become larger during coalescence, while the increase in size of smaller droplets is relatively small. This may explain to some extent the bigger droplet size and the more polydisperse nature of the B series emulsions. Another possibility is that for the B series, the smaller droplets may not be formed in the first place. It has been established that 1% w/w xanthan gum solution lowers the surface tension of water at air/water interface (Prud'homme and Long, 1983). It seems likely that it is surface active at the oil/water interface, and acts as an emulsifier, along with egg yolk, by lowering the interfacial tension. As a result, the smaller droplets became thermodynamically more stable in the A series, while in the B series, the equilibrium droplet size was larger. Xanthan gum was used without purification in the present work. Hence no attempt could be made to establish the cause of the surface activity. Our study indicates that commercial xanthan gum is surface active, which is in agreement with the results obtained by Prud'homme and Long (1983).

There is one difference between the emulsions studied here and the commercial salad cream and mayonnaise systems: no acetic acid was used. The presence of acetic acid in commercial mayonnaise lowers the pH, and strong interactions between the polyelectrolyte molecules (egg yolk proteins and xanthan gum) could be expected, which would have a further effect on the rheology and stability of commercial mayonnaise.

CONCLUSIONS

IN SOYA OIL-WATER EMULSIONS, xanthan contributes to the emulsion stability by two mechanisms. Part of the xanthan is adsorbed at oil/water interface, thus lowering the surface tension and reducing the droplet size, while the remaining part forms liquid crystalline lamellae in the continuous water phase and stabilizes the emulsion by physically trapping the emulsion droplets in the microgel matrix. In 20%, 40% and 60% oil-water emulsions, the yield behav-

ior is primarily due to xanthan which, through the formation of liquid crystalline structure effectively suspends the emulsion droplets. These then may act as filler particles in the composite system. However, in the case of 70% emulsion, there is a small but significant contribution arising from the need to modify the individual droplet deformations when shear is applied to the emulsion.

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A Model for Food Desiccation in Frozen Storage

Q. T. PHAM and J. WILLIX

-----------ABSTRACT-----------

Simple equations were developed to describe the rate of desiccation of unprotected foodstuffs during frozen storage and to describe how the desiccated surface layer of these products affects desiccation rate. These equations indicate that this layer's thermal properties have little effect on the rate of desiccation. Experimental measurements of the resistance of frozen lamb to desiccation were used with these equations to predict desiccation of this product in different environmental conditions. Calculated results agree with measurements from previous surveys and diffusivity measurements. Air velocity should have little effect on desiccation rate once velocity exceeds about 0.1 m/s, whereas radiative heat transfer could have a major effect.

INTRODUCTION

PRODUCT DESICCATION is a major problem in the frozen storage of unprotected foodstuffs such as meat and fish. Although this problem has been surveyed by several workers (for example, Griffith et al., 1932; Kaess, 1961; Kaess and Weidemann, 1961, 1962, 1967a, b, 1969; Cutting and Malton, 1974; Storey and Graham, 1981; Pham et al., 1982), no suitable mathematical model exists to describe the heat and mass transfer processes that occur during desiccation. Considerable research has gone into investigating drying at high temperatures or freeze-drying; however, most of this work is not directly applicable to the desiccation process in frozen storage. This paper presents a simple mathematical description of the desiccation process, with a minimal number of empirical parameters. Such a model can be used to measure the tendency of products to lose water, and to predict the water loss of frozen foodstuffs in different environmental conditions. The method is illustrated using frozen lamb.

THEORY

Desiccation in nonradiative conditions

Much of the water in frozen biological material such as meat or fish is present as pure ice, with an equilibrium vapor pressure equal to that of ice at the same temperature (Storey and Stainsby, 1970; Fennema, 1981). When the ice front coincides with the product's surface, the only resistance to heat and mass transfer to and from the ice is that of the air boundary layer. The following describes the surface conditions in such a case.

Since desiccation is a slow process, the product will be in hygrothermal equilibrium with the air, and pseudo-steady state can be assumed. Under such conditions the ice front is at the wet-bulb temperature. From well-known psychrometric relationships, the heat balance at the surface will be:

(heat used in sublimation) = (heat gained from air)

$$Lk_y(Y_w - Y_a) = h_c(T_a - T_w) \qquad (1)$$

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or
$$\frac{Y_w - Y_a}{T_w - T_a} = -\frac{h_c}{Lk_y} \qquad (2)$$

The transfer coefficients k_y and h_c are related by:

$$\frac{h_c}{k_y} = c_a(Sc/Pr)^a \qquad (3)$$

(In this and subsequent equations, the humid heat of air should be used instead of c_a. However, at subzero temperatures there is little difference between the two, and c_a will be used for convenience, since it is less dependent on temperature and humidity.)

On the psychrometric chart, (T_w, Y_w) is found by drawing a straight line with slope $-s$ through the air conditions (T_a, Y_a) to intersect the saturation curve, where

$$s = h_c/Lk_y = c_a(Sc/Pr)^a/L \qquad (4)$$

The exponent a in Eq. (3) has been determined by various workers. A survey by Kusuda (1965) suggests that a = 2/3, while another by Henry and Epstein (1970) suggests that a = 0.567. Since for the air-water vapor system, (Sc/Pr) has a value of about 0.85, this variation results in an uncertainty of less than 2% in s.

Numerically, the wet-bulb conditions have to be found by an iterative procedure. However, at subzero temperatures an approximate explicit relationship can be derived to avoid iterations or graphical construction. From Fig. 1, by geometry:

$$(s + r)(T_a - T_w) = Y_s - Y_a \qquad (5)$$

where r is the average slope of the saturation curve between T_a and T_w. Also:

$$s(T_a - T_w) = Y_w - Y_a \qquad (6)$$
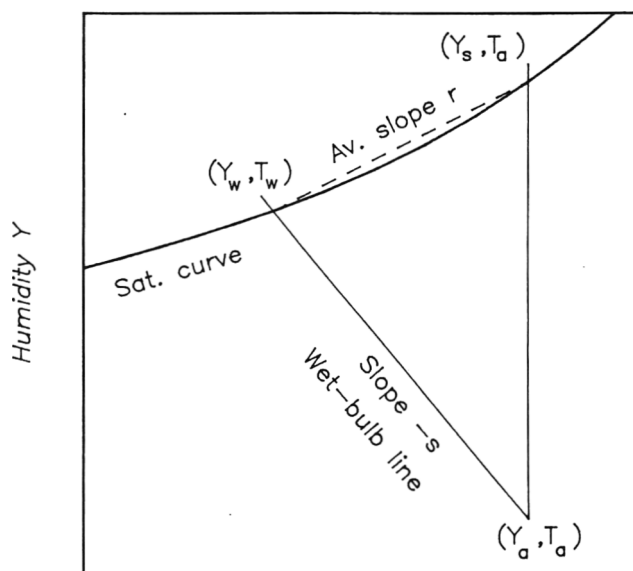
Fig. 1—Linearized psychrometric chart.

By eliminating $(T_a - T_w)$ from Eq. (5) and (6), one gets:

$$Y_w - Y_a = \frac{Y_s - Y_a}{1 + r/s} \tag{7}$$

Since at subzero temperatures $d(r/s)/dT$ is quite small compared to 1, enough accuracy is obtained by calculating r/s at the air temperature T; i.e., r is the slope dY_s/dT at T_a. (At $-5°C$ and 50% RH, the resulting error in $Y_w - Y_a$ is only 2%; it is smaller at lower temperatures and higher RH.)

As desiccation develops, the ice front recedes into the product, leaving behind a porous desiccated layer through which heat and water vapor must diffuse. Thus there is a further resistance R_h to heat transfer and a further resistance R_m to mass transfer. The ice-front temperature will not in general remain the same as the wet-bulb temperature. A heat balance similar to Eq. (1) can be written:

$$\frac{L(Y_i - Y_a)}{1/k_y + R_m} = \frac{(T_a - T_i)}{1/h_c + R_h} \tag{8}$$

or

$$\frac{Y_i - Y_a}{T_i - T_a} = -s' \tag{9}$$

where

$$s' = \frac{1/k_y + R_m}{(1/h_c + R_h)L} \tag{10}$$

$$= \frac{1 + k_y R_m}{1 + h_c R_h} \cdot s \tag{11}$$

By analogy with Eq. (7) we have:

$$Y_i - Y_a = \frac{Y_s - Y_a}{1 + r/s'} \tag{12}$$

$$= \frac{Y_s - Y_a}{1 + \dfrac{r}{s} \cdot \dfrac{1 + h_c R_h}{1 + k_y R_m}} \tag{13}$$

An order-of-magnitude calculation shows that the RHS of Eq. (13) can be further simplified. The term $(1 + h_c R_h)$ represents the relative increase in the resistance to heat

transfer as the ice front recedes into the product, while $(1 + k_y R_m)$ represents the relative increase in the resistance to mass transfer. However, for biological substances (as well as for most other solids), heat conduction is much easier in solids than in air, while mass diffusion is much more difficult. As a result, $(1 + h_c R_h)$ increases much more slowly than $(1 + k_y R_m)$. At low values of $k_y R_m$, $h_c R_h \ll 1$. At high values, $r/s \cdot (1 + h_c R_h)/(1 + k_y R_m) \ll 1$. In both cases the term $h_c R_h$ can be neglected (which is equivalent to assuming that there is no temperature gradient across the desiccated layer). The error in doing this is at most 2%. (A rigorous proof of the validity of this simplification is given in Appendix A.)

Thus, Eq. (13) reduces to:

$$Y_i - Y_a = \frac{Y_s - Y_a}{1 + (r/s)/(1 + k_y R_m)} \tag{14}$$

The rate of weight loss from the product is:

$$\frac{dW}{dt} = \frac{A(Y_i - Y_a)}{1/k_y + R_m} \tag{15}$$

Combining Eq. (14) and (15):

$$\frac{dW}{dt} = \frac{k_y A(Y_s - Y_a)}{1 + r/s + k_y R_m} \tag{16}$$

The numerator on the right-hand side of Eq. (16) is a rate expression. The denominator is a reduction factor accounting for wet-bulb cooling effects (term r/s) and the resistance of desiccated layer ($k_y R_m$).

The quantity r/s is a function of temperature. It is plotted against T in Fig. 2 and can be calculated in the range given ($T < 0°C$) by the equation

$$r/s = e^{0.085 T_a} \tag{17}$$

so that Eq. (16) can be written as:

$$\frac{dW}{dt} = \frac{k_y A(Y_s - Y_a)}{1 + e^{0.085 T_a} + k_y R_m} \tag{16'}$$

When $R_m = 0$, Eq. (16) (or 16') gives the rate of sublimation from ice or a glazed surface. Thus, the desiccated layer slows the sublimation rate by a factor f:

$$f = 1 + k_y R_m/(1 + r/s) \tag{18}$$

$$= 1 + k_y R_m/(1 + e^{0.085 T_a}) \tag{18'}$$

Since several approximations have been introduced to develop the simplified equations above, sample calculations were performed to check that the errors do not accumulate. The simplified equation (Eq. 16') was compared with the original equations (Eq. 8 plus the nonlinearized saturation curve), using data for lamb meat (which will be presented later) and putting $h_c R_h = 0.02 k_y R_m$ in Eq. (8) (see Appendix A). The maximum error in the desiccation rate due to the simplifications in this paper is only about 1.5% at $-1°C$, decreasing to 0.5% at $-15°C$; relative humidity and velocity ranges associated with this error are 0 to 100% and 0 to 1 m/s respectively.

Effect of radiative heat transfer

In the previous section, radiative heat transfer was neglected, which is a reasonable assumption for product surrounded by other products at the same temperature. In other situations, radiative heat transfer is usually important. In such cases it is convenient to define a total or effective heat transfer coefficient,

$$h_e = h_c + h_r \tag{19}$$

and an effective environmental temperature (Keey, 1972)

$$T_e = (h_c T_a + h_r T_r)/h_e \tag{20}$$
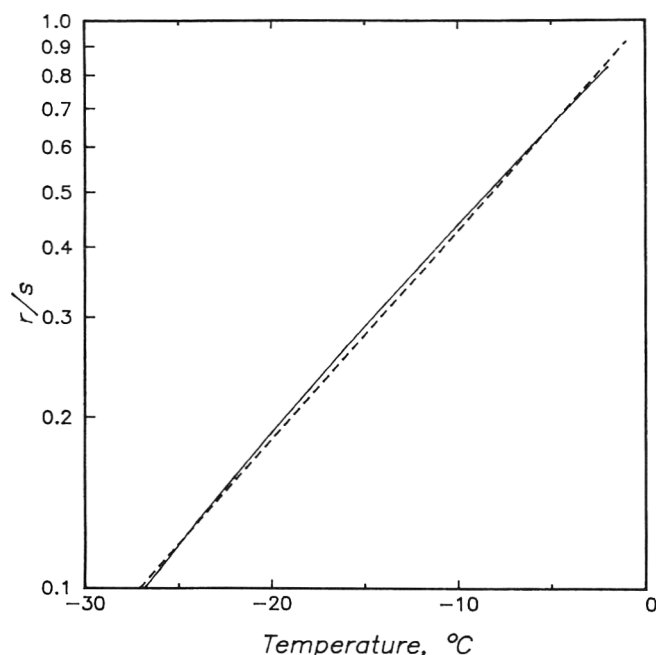
Fig. 2—Plot of r/s vs temperature: —— exact calculation for r/s; ----- Eq. (17).

For a glazed surface, the heat balance at the ice front now becomes:

$$L k_y (Y_w - Y_a) = h_e (T_e - T_{we}) \qquad (21)$$

and in the presence of a desiccated layer:

$$\frac{L(Y_i - Y_a)}{1/k_y + R_m} = \frac{T_e - T_i}{1/h_e + R_h} \qquad (22)$$

which are analogous to Eq. (1) and (8) for the nonradiative case.

Now the derivation carried out in the previous section (Eq. 1 to 18) can again be performed, with h_c, T_a and s replaced by h_e, T_e and s_e respectively, where:

$$s_e = h_e/(L k_y) \qquad (23)$$

$$= \alpha s \qquad (24)$$

where

$$\alpha = 1 + h_r/h_c. \qquad (25)$$

The simplification carried out in Appendix A is still valid since the magnitude of h_r will generally be similar to or less than that of h_c for exposed products.

Thus, the rate of weight loss is given by:

$$\frac{dW}{dt} = \frac{k_y A (Y_{se} - Y_a)}{1 + r/\alpha s + k_y R_m} \qquad (26)$$

$$= \frac{k_y A (Y_{se} - Y_a)}{1 + e^{0.085 T_e}/\alpha + k_y R_m} \qquad (26')$$

In these equations, Y_{se} is the saturation humidity calculated at T_e. When $R_m = 0$, Eq. (26) (or 26') gives the sublimation rate from ice or glazed surfaces. Thus, the reduction in the rate of desiccation due to the desiccated layer is:

$$f = 1 + k_y R_m/(1 + r/\alpha s) \qquad (27)$$

$$= 1 + k_y R_m/(1 + e^{0.085 T_e}/\alpha) \qquad (27')$$

These expressions assume that h_r is the same for ice and for desiccated products, which is usually true since both have emissivities close to unity.

MATERIALS & METHODS

IN THE PREVIOUS SECTION, equations were derived to calculate the rate of desiccation as a function of environmental conditions and the resistance of the desiccated layers to water vapor diffusion, R_m. Conversely, if the rate of desiccation and all the environmental conditions are known, R_m can be determined. An experiment was carried out to do this for frozen lamb. The results will make possible the prediction of weight loss of this material in various situations.

Two freshly frozen lamb carcasses were used, one weighing 13.4 kg with a fairly heavy fat cover (PM grade), and one weighing 11.0 kg with a light fat cover (YL grade). Each carcass was divided into six portions: two forequarters (FQ), two loins and flaps (LF) and two legs. The cut surfaces were sealed with vaseline and polyethylene to prevent sublimation. One cut from each of the six pairs was glazed by being repeatedly cooled to $-35°C$ and immersed in water. All cuts were then exposed to similar environmental conditions (air temperature, velocity and RH) by being hung in a windbox inside an environmental tunnel (Fig. 3). After equilibration the cuts were weighed intermittently over a period of 20-100 hr, using an electronic balance accurate to 0.1g. The cuts were then stored at $-15°C$ for a few weeks and allowed to lose more weight. The test was then repeated. Tests alternated with storage periods for several months.

Under the conditions of the environmental tunnel (radiation from surfaces at air temperature), the rate of desiccation from the glazed and unglazed meat would be described by Eq. (26). Thus, provided the temperatures and humidity are known, k_y (and α) can be obtained from the glazed meat data and R_m from the unglazed meat data.

Environmental conditions

Air temperature. The air temperature was controlled at $-15°C \pm 0.3°C$ for all tests and was measured with copper-constantan thermocouples.

Air velocity. The airflow in the tunnel was controlled with a variable-speed fan. The windbox (Fig. 3) ensured that air velocity around the meat was low and uniform. The air velocity profile was checked with a differential thermal anemometer and found to be flat to within $\pm$ 10%. The total airflow through the windbox was measured by channelling the air through a precalibrated vane anemometer. For most tests air velocity was kept at 0.03 m/s.

Air humidity. Humidity was not controlled but was kept constant during each run by operating at a constant refrigerant temperature. Measurement was a problem as there was no suitable instrument for measuring humidity at subzero temperatures. Therefore, the humidity driving force $(Y_{se} - Y_a)$, which appears in Eq. (26), was measured as follows.

Two copper spheres, 40 mm diameter, were placed in a high-velocity region (2-3 m/s) of the air stream. One contained a heating element and its surface temperature was measured with four thermocouples, whose readings generally differed by no more than 0.25°C at any one time. The other had been glazed by being dipped in liquid nitrogen and then water, to build up a layer of ice 0.5-0.8 mm thick. This sphere was weighed intermittently to an accuracy of 0.01g using an electronic balance (Fig. 3). The airflow was
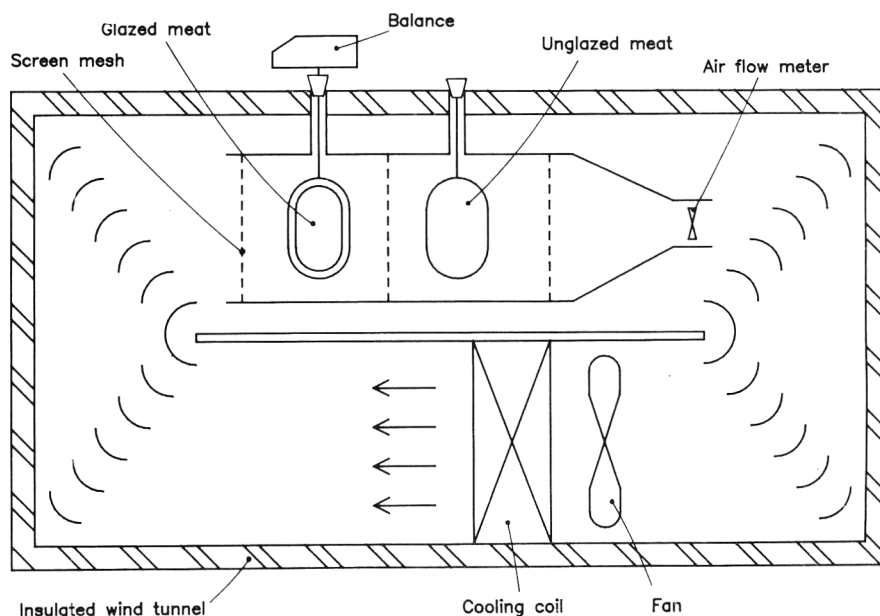
Fig. 3—Experimental set up.

stopped during weighing. When the ice layer started to wear off (as indicated by a fall-off in the rate of weight loss), which took about 20-30 hr, the sphere was reglazed.

From the heat input and the temperature difference between the surface of the copper sphere and the air, the heat transfer coefficient h_c was calculated. The mass transfer coefficient k_y was deduced from the heat/mass transfer analogy (Eq. 4), plus taking into account the slight difference in size between the spheres due to the ice layer. $(Y_{se} - Y_a)$ was then calculated from k_y and the rate of weight loss of the glazed sphere using Eq. (26).

RESULTS

TYPICAL WEIGHT LOSS CURVES for the glazed sphere, the glazed meat and the unglazed meat are shown in Fig. 4. All three curves show good linearity, the correlation coefficients for almost all runs being better than 0.99.

Fig. 5 and 6 show the variation of R_m with the percentage weight loss. The data for legs and LF were plotted together as there was little difference between them. Allowing for the scatter in the data (especially data for the leg and LF from the PM carcass), R_m increased linearly with weight loss, showing that the diffusivity of water vapor was uniform with respect to the depth of the desiccated layer. The linear regression coefficients for R_m vs % weight loss are given in Table 1.
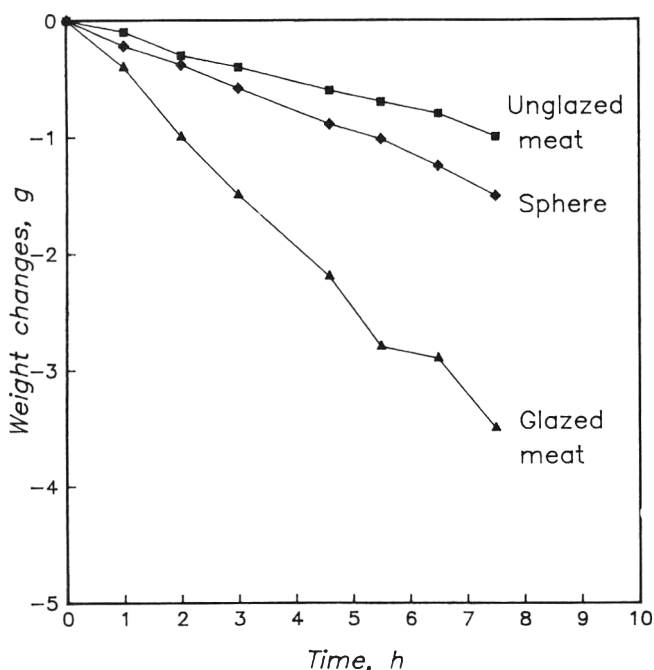
Fig. 4—Typical weight loss curves.
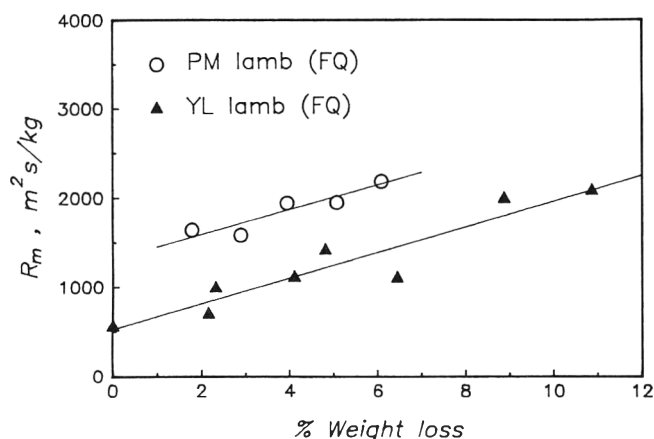
Fig. 5—Variation of R_m with weight loss for lamb forequarters.

Of interest also is the slope of the plot of R_m vs the weight loss per unit area, W/A (the respective intercepts are of course the same as in Table 1). These slopes are given in Table 2.

Using these values and assuming frozen water contents of 42% ("wet" basis) for PM lamb and 52% for YL lamb (Fleming, 1969), the thickness of the desiccated layer and hence the diffusivity D_s of water through meat can be calculated (Eq. A2 of Appendix A). These D_s values, given in Table 2, are rough estimates only since the frozen water content can vary a great deal from the values assumed above.

The D_s values are one order of magnitude lower than the diffusivity of water vapor in air (2.0×10^{-5} m^2/s at $-15°$C). They agree with data from Prendergast (1982), who used diffusion cells to directly measure D_s for lamb meat and found diffusivities in the range 0.5×10^{-6} to 2.5×10^{-6} m^2/s at $-15°$C depending on fat content. The low diffusivity of the LF is attributed to the fact that a significant fraction of its surface is the wall of the body cavity and therefore covered by peritoneum.

APPLICATION: PREDICTION OF WEIGHT LOSSES

Weight loss curve

Values of R_m from experimental data can be substituted in the equations presented earlier (Eq. 16 or 16' for non-radiative situations; Eq. (26) or (26') for situations where radiation is important), and the equations integrated to predict weight loss behavior in any situation.

Table 1—Linear regression of R_m vs percentage weight loss

Carcass grade	Portion	Slope	Intercept	Correlation coefficient
PM	FQ	138	1317	0.943
	Leg & LF	551	141	0.822
YL	FQ	144	525	0.957
	Leg & LF	316	500	0.927
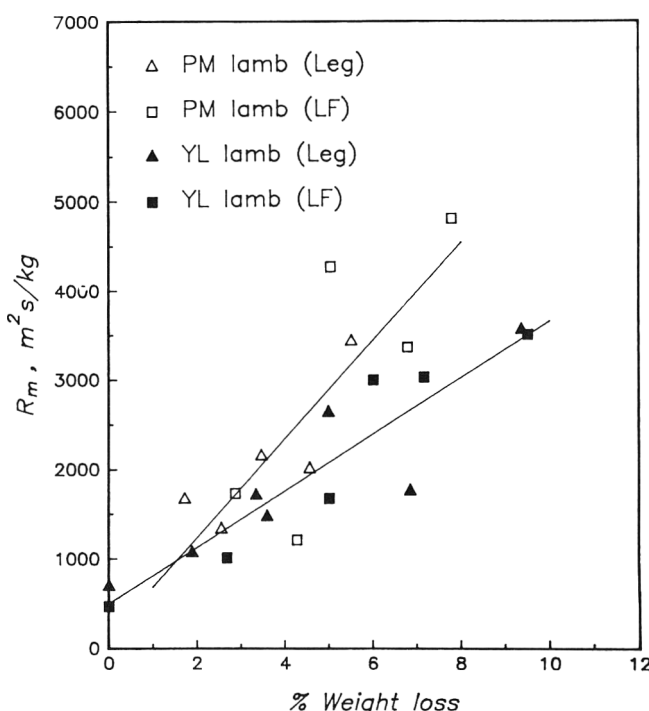
Fig. 6—Variation of R_m with weight loss for lamb legs and loin-and-flaps.

This was done for PM and YL carcasses with the same dimensions and properties as the test carcasses, under the following conditions: Air temperature: −15°C; Radiation temperature: −15°C; Air RH: 90%; Air velocity: 0 and 0.2 m/s.

These are conditions typical of New Zealand cold stores. Zero air velocity occurs in natural-convection-cooled stores or in sheltered spots in forced-draft-cooled sotres. Velocities of 0.2 m/s are common in exposed spots in the latter. As will be seen, air velocities higher than 0.2 m/s have little additional effect on weight loss.

To calculate h_c and k_y, a sphere was used as a model, as the zero-velocity limit of this case is well known (Frössling, 1938; modified by Rowe et al., 1965):

$$Nu = 2 + 0.69 \, Re^{0.5} \, Pr^{0.33} \qquad (28)$$

The diameter of the model sphere was arbitrarily chosen as 0.1m. Its actual diameter (within reason) does not significantly influence the results, because the resistance of meat to mass transfer will in all cases be the overriding factor, the resistance of the air ($1/k_y$) playing only a minor role except in the initial stages of desiccation. Also, the use of a sphere as a model should not affect the results since Pasternak and Gauvin (1960) and Skelland and Cornish (1963) showed that, providing an appropriate value of the characteristic dimension is taken, the actual shape is of minor significance.

Fig. 7 and 8 show the calculated weight loss curve for each carcass, together with data from Pham et al. (1982), who weighed pallets of YL- and PM-grade lamb carcasses kept in meat-works' cold stores over a period of up to 10 months. (These data were obtained at temperature slightly different from −15°C, and have been corrected for temperature effects, using Pham et al.'s log-linear regression curve; i.e., the rate of weight loss is assumed to increase by 0.78% for each °C of temperature rise. The relative humidity in the cold stores were not measured at the time of the survey, but subsequent measurements suggested they would be in the range 85–95%.)

The calculated rate of weight loss fell off with time, but after the first month or two the curvature was slight, es-

pecially for the zero-velocity case. This explains why Pham et al. (1982) observed a linear relationship between weight loss and time after the first month of frozen storage. Their data lie within the zero and "high" velocity bounds in Fig. 7 and 8.

Effect of air velocity

Fig. 9 shows the effect of air velocity on the weight loss after 6 months (henceforth called "WL6"). Above about 0.1 m/s, WL6 changes very little. When other models were used to calculate h_c and k_y (spheres of twice or half the diameter of 0.1 m), the shape of the curves was virtually unchanged from those in Fig. 9, showing that within reason the geometrical size used is not important. Thus unless the air velocity is reduced to almost zero, variations in its value have little direct effect on the rate of desiccation.

Effect of air temperature

Fig. 10 shows the effect of air temperature on WL6. Also shown are data from Cutting and Malton (1974) and Pham et al. (1982). These data are rather scattered due to the many uncontrolled factors in the environmental conditions and materials used. For the latter set of data only results from newer single-story cold stores are shown, since Pham et al. reported that results from older multistory stores were subject to large unpredictable variations, due to

Table 2—Slope of R_m (in m^2s/kg) vs weight loss per unit area (W/A) (in kg/m^2) and estimated diffusivity of water vapor through desiccated lamb meat at −15°C

Carcass grade	Portion	Surface area, m^2	Initial wt, kg	$\dfrac{dR_m}{d(W/A)}$	D_s, $10^{-6}m^2/s$
PM	FQ	0.118	3.11	525	3.3
	Leg	0.078	2.13	2030	0.9
	LF	0.173	1.56	6130	0.3
YL	FQ	0.087	2.03	617	2.3
	Leg	0.074	1.93	1213	1.2
	LF	0.145	1.56	2943	0.5
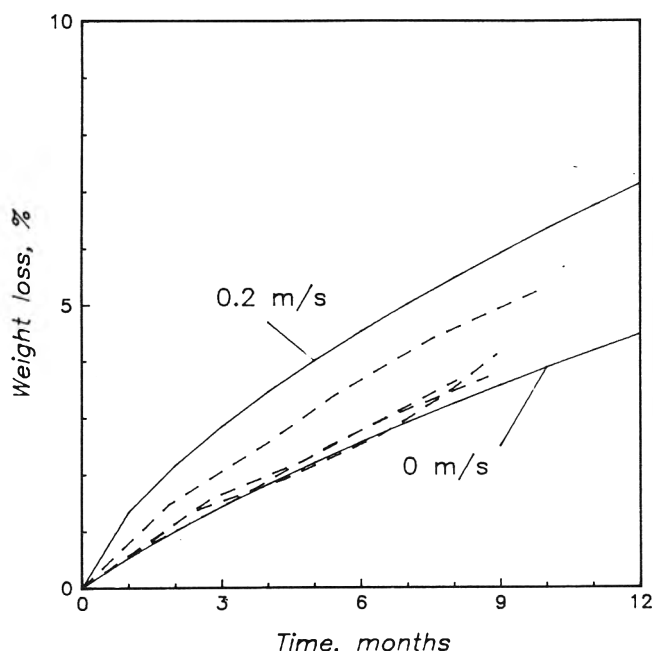
Fig. 7—Weight loss curves for PM lamb: —— calculated at −15°C, 90% RH and 0 or 0.2 m/s air velocity; ----- data from Pham et al. (1982).
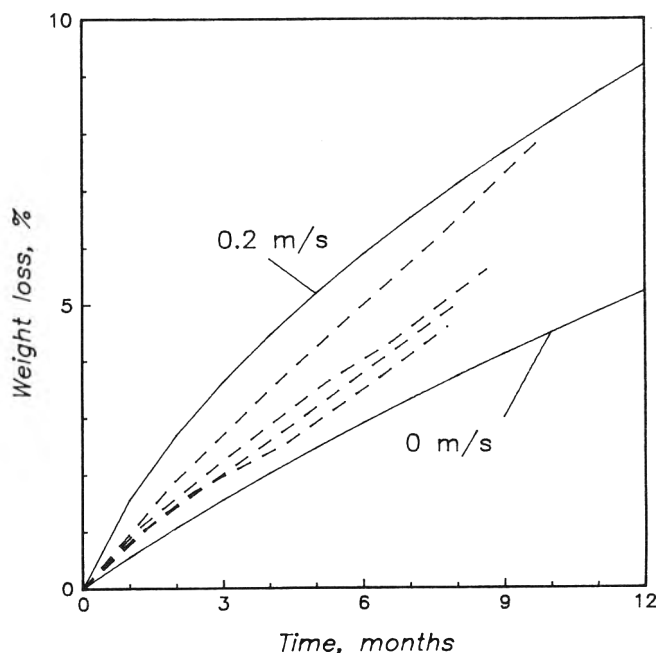
Fig. 8—Weight loss curves for YL lamb: —— calculated at −15°C, 90% RH and 0 or 0.2 m/s air velocity; ----- data from Pham et al. (1982).

local temperature differences. Good agreement is obtained; the calculated values enclosed most of the experimental data. Since the R_m values were obtained at a single temperature ($-15°C$), R_m was assumed to vary with temperature in the same way as the inverse of water vapor diffusivity in air; i.e., it would decrease by 0.7% for each $°C$ temperature rise.

Effect of relative humidity

Fig. 11 shows the effect of relative humidity on WL6. Because the resistance of the desiccated meat increases with time, WL6 does not increase linearly with (100-RH) but increases more slowly.

Effect of radiating temperature

Fig. 12 shows what happens when the product was surrounded by a radiating surface. The effect is very significant, even for quite small temperature differences between the radiating surface and the air, especially at zero air velocity. T_r can be lower than T_a when, say, the carcass is near a (natural-convection) cooling coil. It is higher when the carcass is near a wall, floor or light, and values of 1 to $2°C$ for $(T_r - T_a)$ are quite common (Pham et al., 1982). Both zero velocity and highly variable values of $(T_r - T_a)$ are more commonly found in older natural-convection-cooled store than in newer forced draft stores. This is why the former type of stores shows a very large range of varia-

Fig. 9—Effect of air velocity on 6-month weight loss of PM and YL lamb at $-15°C$ and 90% RH.
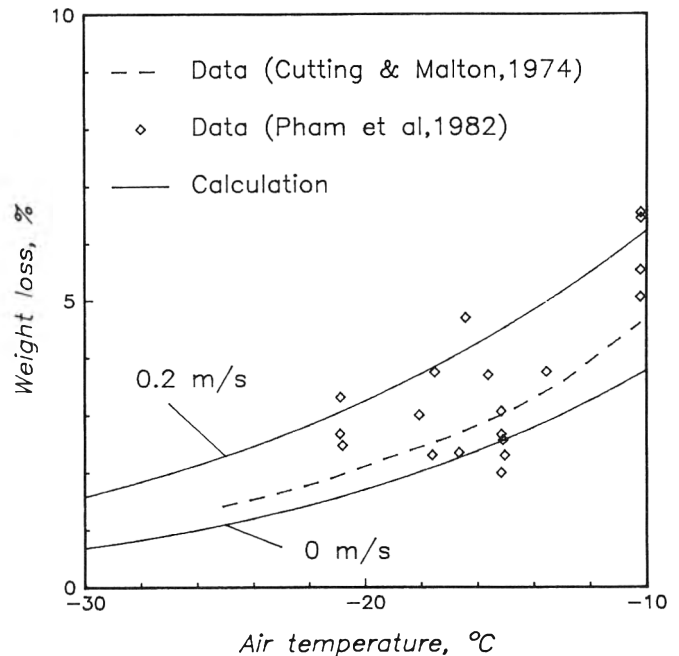
Fig. 10—Effect of air temperature on 6-month weight loss of PM lamb at 90% RH and 0 or 0.2 m/s air velocity.
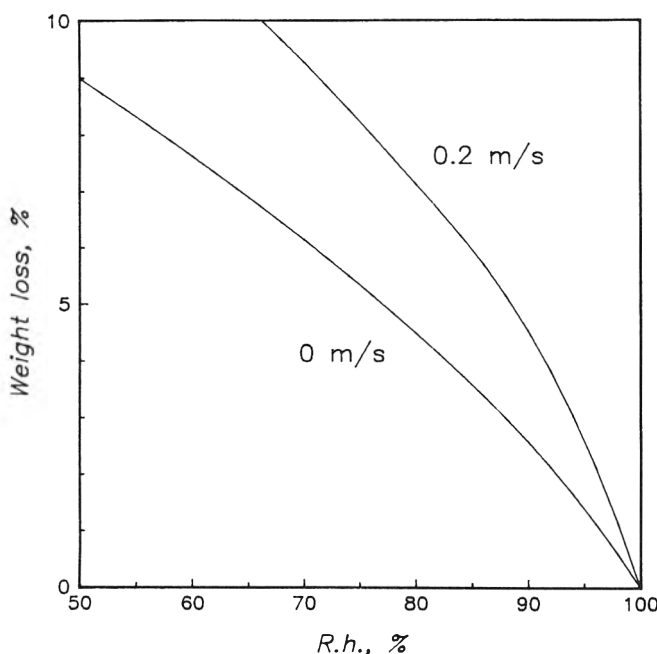
Fig. 11—Effect of RH on 6 month weight loss of PM lamb at $-15°C$ and 0 or 0.2 m/s air velocity.
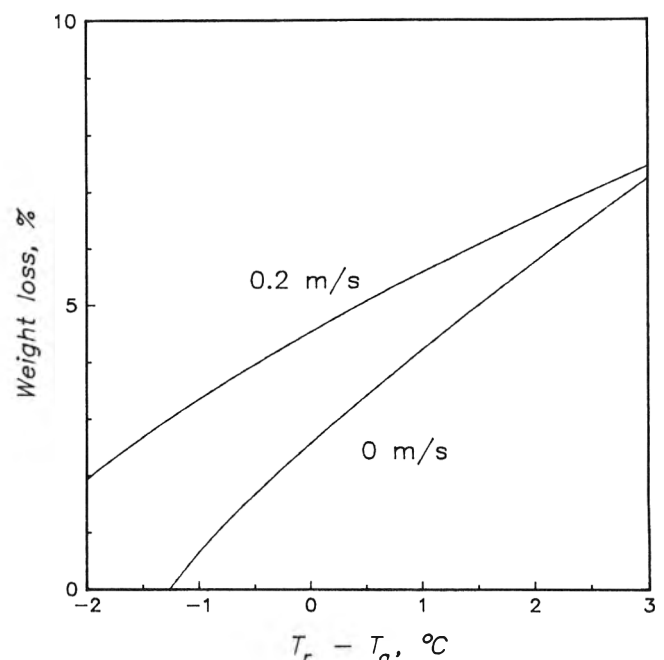
Fig. 12—Effect of radiating temperature on 6 month weight loss of PM lamb at 90% RH, $-15°C$ and 0 or 0.2 m/s air velocity.

tion (20:1) in weight loss, even in the same building, while the range for the latter type of stores is much less (2:1).

CONCLUSIONS

MUCH OF THE THEORY presented in this paper is basic psychrometry and drying theory. However, at sub-zero temperatures, certain simplifications are possible (Eq. 7, 12 and Appendix A), leading to very simple expressions for the rate of weight loss (Eq. 16 or 16' and 26 or 26') and the reduction in the desiccation rate due to the presence of a desiccated layer on the surface of the product (Eq. 18 or 18' and 27 or 27'). The rate equations consist of an elementary rate expression, $k_y A(Y_s - Y_a)$, reduced by a factor $(1 + r/s + k_y R_m)$ where the term r/s accounts for wet-bulb cooling effects and $k_y R_m$ accounts for the resistance of the desiccated layer. The resistance to water vapor diffusion of the desiccated layer (R_m) is an important factor, but not its resistance to heat conduction (R_h). This simplifies the situation considerably and enables R_m to be readily measured. Once R_m is known, the desiccation behavior of the product can be predicted. The term r/s is adequately represented by an exponential expression, $e^{0.085T}$.

Results based on this model have confirmed previous survey data and provide an insight into the mechanism and causes of weight loss in cold storage. Among the more significant findings are: (1) Above about 0.1 m/s the rate of weight loss is unaffected by air velocity; (2) Radiation heat transfer can have a major effect on weight loss, especially at zero air velocity.

NOTATION

a   Exponent in Eq. (3)
A   Exposed surface area of product, m^2
b   Thickness of desiccated layer, m
c   Specific heat, J/kg K
d   Length scale, m
D   Diffusivity of water vapour through air, m^2/s
D_s Diffusivity of water vapour through desiccated product, m^2/s
e   Relative error (Eq. A8)
f   Reduction factor in desiccation rate, compared with ice
h_c Convection heat transfer coefficient, $W/m^2 K$
h_e Effective or total heat transfer coefficient ($h_c + h_r$), $W/m^2 K$
h_r Radiation heat transfer coefficient, $W/m^2 K$
K   Constant defined in Eq. A6
k_a Thermal conductivity of air, W/mK
k_s Thermal conductivity of desiccated product, W/mK
k_y Mass transfer coefficient, $kg/m^2 s$
L   Latent heat of sublimation, J/kg
Nu   Nusselt number, $h_c d/k_a$
Pr   Prandtl number of air, $c_a \mu_a/k_a$
r   Local gradient of water vapour saturation curve, K^{-1}
R_h Resistance of desiccated product layer to heat conduction, $m^2 K/W$
R_m Resistance of desiccated product layer to water vapour diffusion, $m^2 s/kg$
Re   Reynolds number, $dv\rho_a/\mu_a$
s   Absolute value of slope of wet-bulb line (Eq. 4), K^{-1}
s'   Absolute value of slope of ice-front line (Eq. 10), K^{-1}
Sc   Schmidt number of the air-water vapour system ($\mu_a/\rho_a D$)
t   Time, s
T   Temperature, °C
T_e Effective environmental temperature, $(h_c T_a + h_r T_r)/h_e$, °C
T_r Temperature of radiating surface, °C
v   Air velocity, m/s
W   Product weight change, kg
Y   Humidity, kg/kg
Y_s Saturation humidity at T_a, kg/kg
Y_{se} Saturation humidity at T_e, kg/kg
α $1 + h_r/h_c$
μ Viscosity, kg/m s
ρ Density, kg/m^3

Subscripts

a   Air
e   Effective (i.e., including radiative effects)
i   Ice front
w   Wet-bulb (or ice bulb)

APPENDIX A

Proof of Eq. (14)

The resistances to heat and mass transfer, respectively, of the desiccated layer can be expressed as:

$$R_h = b/k_s \qquad (A1)$$

$$R_m = b/D_s \rho_a \qquad (A2)$$

Hence:

$$R_h = D_s \rho_a R_m /k_s \qquad (A3)$$

The heat and mass transfer coefficients are related by Eq. (3) in the text

$$h_c = c_a (Sc/Pr)^a k_y \qquad (A4)$$

Thus

$$h_c R_h = (D_s \rho_a R_m /k_s)\, [c_a (Sc/Pr)^a k_y]$$

$$= \frac{D_s \rho_a c_a (Sc/Pr)^a}{k_s} R_m k_y$$

$$= K\, R_m k_y \qquad (A5)$$

where

$$K = D_s \rho_a c_a (Sc/Pr)^a /k_s \qquad (A6)$$

Hence the denominator on the RHS of Eq. (13) in the text can be written as:

$$\text{Denom.} = 1 + \frac{r}{s} \cdot \frac{1 + h_c R_h}{1 + k_y R_m} = 1 + \frac{r}{s} \cdot \frac{1 + K k_y R_m}{1 + k_y R_m} \quad (A7)$$

Now consider what error will be involved by ignoring the term ($K k_y R_m$). The relative error e in "Denom." will be:

$$e = \frac{K k_y R_m \cdot (r/s)/(1 + k_y R_m)}{1 + \frac{r}{s} \cdot \frac{1 + K k_y R_m}{1 + k_y R_m}} \qquad (A8)$$

Taking derivative:

$$\frac{de}{d(k_y R_m)} = \frac{(1 + r/s)\,(r/s)\, K}{[1 + r/s + (1 + Kr/s)k_y R_m]^2}$$

$$> 0$$

Hence the relative error always increases with $k_y R_m$ and is largest as $k_y R_m$ tends to infinity (i.e., as desiccation becomes more severe). At infinite values of $k_y R_m$ the error is then, from Eq. A8:

$$e_{max} = \frac{Kr/s}{1 + Kr/s} \qquad (A10)$$

At sub-zero temperatures: $c \cong 1000$ J/kg K; $\rho \cong 1.4$ kg/m^3; $(Sc/Pr)^a \cong 0.9$.

Also, experimental measurements (Prendergast, 1982; this paper) give values of D_s of the order 10^{-6} m^2/s, while k_s is about 0.06 W/mK for desiccated frozen meat (Harper

Factors Governing Lysinoalanine Formation in Soy Proteins

MENDEL FRIEDMAN, CAROL E. LEVIN, and AMY T. NOMA

─────────── ABSTRACT ───────────

Exposing soy protein to alkaline conditions (pH 8-14) for various time periods (10-480 min), and temperatures (25-95°C at 10°C intervals) destroyed all of the cystine and part of arginine, lysine, serine, and threonine residues. These losses were accompanied by the appearance of lysinoalanine (LAL) and unidentified ninhydrin-positive compounds. Acylation of the proteins minimized LAL formation. Addition of cysteine, copper salts, dimethyl sulfoxide and glucose also suppressed LAL production. Oxygen, with one possible exception, did not affect LAL formation. Free and protein-bound lysinoalanine is stable to acid but not basic conditions used for protein hydrolysis. Mechanisms are described to explain the observed influence of these variables on LAL generation.

INTRODUCTION

CROSSLINKED AMINO ACIDS have been identified in acid hydrolyzates of alkali-treated proteins. One such cross-linked amino acid, lysinoalanine (LAL) causes histological changes in rat kidneys characterized by enlargement of the nucleus and cytoplasm, increased nucleoprotein content, and disturbances in DNA synthesis and mitosis. The lesions affect epithelial cells of the straight portion (*pars recta*) of the proximal renal tubules. (Woodard et al., 1975; Gould and MacGregor, 1977; Sternberg and Kim, 1977; Karayannis et al. 1979 a, b; Finot, 1983). For recent reviews on LAL and related aspects of protein-alkali reactions, see Whitaker and Feeney (1983), Friedman, (1982a), and Friedman et al. (1984a).

These observations cause some concern about the nutritional quality and safety of alkali-treated foods. Chemical changes that govern the formation of unnatural amino acids need to be studied and explained, and strategies to minimize or prevent these reactions need to be explored.

In this paper we extend our previous studies (Cavins and Friedman, 1967; Finley et al., 1978; Friedman, 1973, 1977, 1978a, b; Friedman et al., 1977, 1981, 1982, 1984b; Masri and Friedman, 1982; Masters and Friedman, 1979, 1980) by reporting on (a) the hydrolytic stability of LAL, (b) the degradation of alkali-labile amino acid residues in soy protein and the accompanying formation of LAL and new ninhydrin positive compounds as a function of pH, time and temperature of treatment, in the presence of oxygen, carbohydrates, metal salts, sulfhydryl compounds, and dimethyl sulfoxide; and, (c) effects of acylation of amino groups.

MATERIALS & METHODS

Alkali-treated soy protein: general procedure

Soy protein (0.5g; Promine-D, U.S. Biochemical Co., Cleveland, OH) was suspended in 50 mL 0.1N NaOH in a 125 mL Erlenmeyer flask. After initial pH was measured, the flask was stoppered with a Nalgene cap and covered with aluminum foil to prevent the top from popping. The flask was then placed into a water bath at the appropriate temperature. At the end of the treatment, the flask was cooled under running water and the pH measured. The contents were immediately dialyzed against dilute acetic acid (0.25%) for one day, and thereafter against distilled water for two days. The samples were then lyophilized.

Variations in procedure

pH Study. A 0.05M borate buffer of specified pH was used instead of 0.1N NaOH.

Modified proteins. Acetylated and succinylated soy proteins were prepared as described previously (Friedman, 1978a, b; 1982b). The extent of acylation or succinylation was approximately 80%, as determined by the manual ninhydrin procedure (Friedman et al., 1984c).

Effect of oxygen. Oxygen or nitrogen (oxygen-free) was bubbled through the samples for 4 min. The reaction flasks were immediately stoppered with greased ground-glass stoppers held in place with rubber bands.

Effect of glucose. Varying amounts of glucose were added to the soy protein suspensions.

Metal salt study. The appropriate metal salt (0.5 mmoles) was added to the protein suspension.

Effect of dimethyl sulfoxide (DMSO). The soy protein (0.5g) was suspended in a mixed solvent consisting of 25 mL 0.1N NaOH and 25 mL DMSO.

Amino acid analyses

Amino acids were analyzed on a single-column Durrum 500 amino acid analyzer, as previously described (Friedman et al., 1981). In this system, lysinoalanine is eluted just before histidine. The color constant of LAL was determined by comparison with an authentic sample purchased from Miles Laboratories (Elkhart, IN). Serine D/L ratios were determined by a gas chromatographic procedure (Tovar and Schwass, 1983).

RESULTS & DISCUSSION

THE INFLUENCE of a series of parameters expected to govern LAL formation in soy proteins was explored to define the scope of the reaction.

Factors favoring lysinoalanine formation

pH of treatment. The initial and final pH values differed only slightly. The soy protein usually completely dissolved after exposure to alkali for a few minutes. The amino acid composition, in terms of g/16g N, of soy protein solutions adjusted to the appropriate pH ranging from 8-14 and held at 75°C for 3 hr is listed in Table 1. The following amino acids were affected during the alkaline treatment: cystine, serine, threonine, arginine, and lysine. Since all of the cystine residues were lost at pH 9, they seem to be the most labile to alkali. The lysine content of the hydrolyzates began decreasing above pH 9, serine and threonine content near pH 12, and arginine content near pH 12.5. These changes were accompanied by the appearance of LAL, which began forming at pH 8 and continuously increased with pH up to pH 12.5; and then decreased at pH 14. Thus, in terms of g/16g N, LAL content increased from 0.17 at pH 8 to 0.50 at pH 9; 0.89 at pH 10; 1.13 at pH 11; 1.63 at pH 12; 3.18 at pH 12.5; and 2.45 at pH 14, a decrease from the previous pH.

It should be emphasized that when amino acid values are reported in g/16g N, the fraction that is degraded during

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Table 1—Effect of pH on the amino acid composition of soybean protein[a] (Values are in g/16g N)

Amino acid	Dialyzed control	pH						
		8.0	9.0	10.0	11.0	12.0	12.5	14.0
ASP	12.44	12.10	12.27	12.26	12.52	13.57	13.08	15.10
THR	3.63	3.55	3.57	3.77	3.62	3.88	2.99	0.92
SER	5.39	5.28	5.32	5.40	5.32	5.57	4.01	1.25
GLU	17.86	18.05	18.24	18.88	18.87	20.23	19.66	23.47
PRO	4.91	4.94	4.92	5.14	5.07	5.58	5.18	5.20
GLY	3.96	3.96	3.92	4.07	4.08	4.40	4.53	3.92
ALA	3.99	3.85	3.84	4.02	4.06	4.42	4.53	4.34
CYS	0.71	0.34	0.00	0.00	0.00	0.12	0.00	0.00
VAL	4.62	4.60	4.62	4.87	4.94	5.36	5.62	5.97
MET	0.25	0.16	0.00	0.79	0.53	0.73	1.27	0.24
ILEU	4.40	4.43	4.45	4.66	4.70	5.03	5.13	4.34
LEU	8.08	7.88	7.97	8.30	8.41	9.14	9.53	10.15
TYR	3.63	3.43	3.41	3.73	3.77	4.09	3.91	3.89
PHE	5.25	5.14	5.25	5.45	5.54	5.97	6.04	6.12
HIS	2.56	2.50	2.55	2.62	2.65	2.74	2.80	2.84
LYS	5.93	5.71	5.52	5.50	5.30	5.34	3.98	6.06
ARG	7.34	7.20	7.34	7.58	7.57	7.87	6.80	3.42
LAL		0.17	0.50	0.89	1.13	1.63	3.18	2.45
X1						0.15[b]	0.48[b]	2.68[b]
X2								1.32[c]
X3								1.06[d]

[a] Conditions: 0.5g protein in 50 mL 0.05M sodium borate, 75°C; 3 hr.
[b] Elutes before LYS
[c] Elutes between MET and ILEU
[d] Elutes before LAL.

Table 2—Stability of free lysinoalanine to acid and base hydrolysis[a]

Time (hrs)	6N HCl[a]	1N NaOH[b]	4.2N NaOH[c]	3.96M Ba(OH)[d]
0	4.69	1.20	1.20	0.91
4	4.36	1.21	1.20	—
8	4.39	1.10	1.25	—
24	4.60	1.05	0.99	0.57
48	4.40	—	—	—
72	4.57	—	—	—

[a] Conditions: 4.69 μmoles LAL and 5.27 μmoles LEU; 10 mL 6N HCl; 110°C. Leucine was used as an internal standard. pH at 100°C = -0.61.
[b] 1.20 μmoles LAL and 1.70 μmoles LEU; 3 mL 1N NaOH; sealed tube; 110°C. pH at 100°C = 11.5.
[c] 1.20 μmoles LAL and 1.70 μmoles LEU; 0.6 mL 4.2N NaOH; 110°C. (Hugli and Moore, 1972). pH at 100°C = 11.7.
[d] 0.91 μmoles LAL and 1.75 μmoles LEU; 5 mL 3.96N Ba(OH)₂; 110°C (Knox et al., 1970). pH at 100°C = 11.9.

Table 3—Stability of added lysinoalanine to acid and base hydrolysis[a]

Solvent	% Recovery lysinoalanine in the presence of:	
	Soy flakes	Starch
6N HCl	90.8	87.7
1N NaOH	68.2	47.6

[a] Conditions: 5.08 μM lysinoalanine and 5 mg soy flakes or starch in 10 mL 6N HCl or 1N NaOH were hydrolyzed in evacuated, sealed tubes for 24 hr at 110°C.

exposure to alkali or lost during dialysis of small molecular-weight fragments will not be included in the results based on N. Consequently, the remaining amino acid values may differ somewhat from the untreated control. The results in Tables 1, 4, and 5 show that this is true for samples treated at the higher pHs, temperatures, and times. Another factor which can affect the apparent results is the formation of new ninhydrin-positive compounds whose elution positions on the amino acid chromatogram may overlap some of the standard amino acids. Finally, since a separate performic acid procedure is needed for accurate analysis of sulfur amino acids (Moore, 1963), the reported values for cystine and methionine, which are based on direct analysis, may not reflect the true content of these amino acids.

Table 1 also shows that the acid hydrolyzate of the pH 12 sample contained a new, ninhydrin-positive compound, designated as X1 (0.15g/16g N). The pH 12.5 and 14 samples contained 0.48 and 2.68 g/16g N of X1, respectively. The pH 14 sample contained two additional compounds, designated as X2 and X3, in amounts of 1.32 and 1.06 g/16g N, respectively (Table 1).

Apparently, LAL was both formed and destroyed during the alkaline treatment used. To obtain evidence for this hypothesis, the hydrolytic stability of pure and protein-bound LAL was examined under acidic and basic condi-

tions. The results, summarized in Tables 2 and 3 and additional studies, show that (a) free LAL appeared to be stable to acid hydrolysis conditions, although the small decrease in LAL may be outside the experimental error; (b) protein-bound LAL in previously described lactalbumin, soy protein, and wheat gluten (Friedman, 1978a, b ; 1979) was stable under acid protein hydrolysis conditions (6N HCl; 110°C; sealed tube) up to 72 hr; (c) exposure of free LAL in a sealed tube to 1N NaOH gave the following time-dependent recovery; 4 hr, 100%; 8 hr, 91.8%; 24 hr, 87.5%; (d) exposure in a sealed tube to 4.2N NaOH under conditions used for tryptophan analysis (Hugli and Moore, 1972; Friedman et al., 1984b) gave the following time-dependent recovery of LAL: quantitative recovery for the 4 and 8 hr runs and 82.4% for the 24 hr run; (e) exposure of free LAL in a sealed tube to 3.96M Ba(OH)₂ for 24 hr under conditions used for tryptophan analysis (Knox et al., 1970) resulted in a 62.5% recovery; (f) LAL in the three proteins mentioned above was completely destroyed when the hydrolysis was carried out in 1N NaOH, even after 4 hr; and (g) during co-hydrolysis with soy flakes or starch, about 10% of the added LAL was destroyed in acid and 32 to 52% in base (Table 3; see also, Raymond, 1980).

We noted that the alkaline hydrolysis conditions induce gelatinization of carbohydrates, especially starch. Such gelatinizations may affect the recovery of LAL by modifying its reactivity with the carbohydrates during hydrolysis.

These results suggest that both formation and destruction of LAL will be catalyzed by strong alkali.

Table 4—Effect of temperature on the amino acid composition of alkali-treated soybean protein[a] (Values are in g/16g N)

Amino acid	Dialyzed control	Temperature of treatment							
		25°C	35°C	45°C	55°C	65°C	75°C	85°C	95°C
ASP	12.44	12.49	12.75	12.36	12.98	13.39	13.95	14.52	13.90
THR	3.63	3.68	3.88	3.59	3.79	3.51	3.16	2.63	1.63
SER	5.39	5.39	5.50	5.21	5.25	4.90	4.15	3.39	2.02
GLU	17.86	18.78	19.29	18.36	19.21	19.96	20.38	20.61	20.31
PRO	4.91	5.14	5.23	4.99	5.24	5.35	5.47	5.51	5.20
GLY	3.96	4.07	4.16	3.97	4.21	4.45	4.77	5.15	5.30
ALA	3.99	4.06	4.16	3.99	4.28	4.50	4.80	5.09	5.04
CYS	0.71	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
VAL	4.62	4.91	4.95	4.78	5.14	5.55	6.05	6.29	6.41
MET	0.25	0.10	0.25	0.24	0.55	0.67	0.89	0.03	0.40
ILEU	4.48	4.57	4.73	4.53	4.79	4.50	5.46	5.62	5.42
LEU	8.08	8.14	8.39	8.07	8.75	9.20	10.00	10.73	10.59
TYR	3.63	3.52	3.73	3.57	3.87	4.12	4.31	4.54	4.20
PHE	5.25	5.36	5.52	5.26	5.67	5.93	6.31	6.66	6.57
HIS	2.56	2.58	2.66	2.51	2.64	2.77	2.76	2.88	2.83
LYS	5.93	5.66	5.66	5.20	5.17	4.90	4.65	4.61	4.72
ARG	7.34	7.45	7.66	7.21	7.47	7.39	6.90	5.84	3.38
LAL	0.00	0.49	0.71	0.96	1.39	2.00	2.49	3.07	2.85
X1					0.12[b]	0.33[b]	0.64[b]	1.09[b]	2.02[b]
X2							0.24[c]	0.37[c]	0.57[c]
X3									0.53[d]

[a] Conditions: 0.5g protein in 50 mL 0.1N NaOH; 3 hr.
[b] Elutes before LYS;
[c] Elutes between MET and ILEU;
[d] Elutes before ASP.

Table 5—Effect of time of treatment on amino acid composition of alkali-treated soybean protein (Values are in g/16g N)

Amino acid	Dialyzed control	Time (min)				
		10	30	180	300	480
ASP	12.44	12.76	12.02	13.47	13.60	14.90
THR	3.63	3.68	3.64	3.31	3.03	2.97
SER	5.39	5.43	5.35	4.45	4.05	3.66
GLU	17.86	19.11	18.95	18.39	18.85	20.03
PRO	4.91	5.06	5.02	4.94	5.23	5.30
GLY	3.96	4.12	4.15	4.38	4.67	5.03
ALA	3.99	4.13	4.20	4.48	4.68	5.10
CYS	0.71	0.00	0.00	0.11	0.00	0.19
VAL	4.62	4.90	5.01	5.49	5.96	6.55
MET	0.25	0.43	0.56	0.56	0.03	0.89
ILEU	4.48	4.74	4.70	5.02	5.29	5.66
LEU	8.08	8.47	8.52	9.42	10.12	10.99
TYR	3.63	3.78	3.85	4.12	4.43	4.62
PHE	5.25	5.55	5.57	5.93	6.30	6.89
HIS	2.56	2.70	2.72	2.71	2.77	2.94
LYS	5.93	5.66	5.25	4.41	4.29	4.36
ARG	7.34	7.60	7.38	6.64	6.53	6.23
LAL		0.75	1.64	2.41	2.79	3.45
X1				0.48[b]	0.66[b]	0.96[b]
X2				0.16[c]	0.28[d]	0.32[c]
X3						0.29[e]

[a] Conditions: 0.5g protein in 50 mL 0.1N NaOH; 75°C.
[b] Elutes before LYS.
[c] Elutes between MET and ILEU.
[d] Elutes before ASP.
[e] Elutes after HIS.

Effect of temperature. In a 1% solution of soy protein exposed to 0.1N NaOH (pH 12.5) for 3 hr at temperatures from 25-95°C, the following amino acids were progressively degraded: cystine, lysine, threonine, serine, and arginine (Table 4). All cystine was destroyed at 25°C; lysine destruction began near 45°C; serine and threonine between 65 and 75°C; and arginine near 75°C. LAL began to appear at 25°C (0.49 g/16g N), continuously increased up to 85°C (3.07 g/16g N), and then decreased at 95°C (2.85 g/16g N).

As with pH, increasing temperature induced formation of an unidentified new, ninhydrin-positive compound eluting before lysine. Its concentration (in g/16g N) increased from 0.12 at 55°C to 0.33 at 65°C; 0.64 at 75°C; 1.09 at 85°C; and 2.02 at 95°C. The 95°C sample also contains two additional new compounds designated as X2 and X3 (Table 4).

Effect of time of treatment. Heating a 1% solution of soy protein in 0.1N NaOH at 75°C for periods ranging from 10-480 min induced a progressive increase in LAL formation and the appearance of new, ninhydrin-positive compounds similar to those mentioned for the pH and temperature treatment (Table 5). Significant amounts of LAL are formed even after only 10 min. Thus, significant amounts of LAL appear to be formed in protein under relatively mild conditions of pH, time, and temperature, such as might be experienced in food processing. Consequently, preventive measures against LAL formation may be justified even if proteins are subjected to mild alkaline conditions during food processing.

Effect of oxygen. LAL values from duplicate experiments in terms of weight % (g/100g sample), mole % (moles per total moles accounted for); and g/16g N for soy protein treated for 1 or 3 hr at 65 and 75°C in 0.1N NaOH saturated with O_2 or N_2 are shown in Table 6. A statistical analysis of the results (Duncan, 1975) from this initial study shows no significant differences between the nitrogen and oxygen samples with one possible exception. The nitrogen atmosphere yielded LAL values for the 65°C − 1 hr samples which were significantly higher at the 0.05 probability level than the corresponding samples treated in an oxygen atmosphere.

Oxygen can, in principle, oxidize protein disulfide bonds to several intermediate oxidation states ending in negatively charged cysteic acid side chains (Friedman, 1973). Because of charge repulsion between the $-SO_3^-$ and OH^- ions, such side chains are expected to form dehydroalanine (the LAL precursor) less readily than disulfide groups (see below). The presence of oxygen may diminish LAL formation by catalyzing such oxidations (Finley and Kohler, 1979). However, because traces of copper and iron salts strongly enhance the action of oxygen on sulfur amino acids (Friedman, 1973; Boonvisut et al., 1982), varying results may be expected, depending on the presence or absence of such trace elements. Moreover, trace-element-catalyzed oxidation of sulfur amino acids by oxygen is accompanied by the

formation of hydrogen peroxide (Feldman et al., 1982), which may be the actual oxidizing agent. In addition to sulfur amino acids, peroxides and persulfates may also oxidize other sensitive amino acid residues, such as histidine, tryptophan, and tyrosine (Hopkins and Spikes, 1970).

Mechanism of lysinoalanine formation

A postulated mechanism of LAL formation shown in Fig. 1 is at least a two-step event (Friedman, 1977, 1982a; Gross, 1977; Whitaker and Feeney, 1978). First, hydroxide ion-catalyzed β-elimination reactions of serine, threonine, cystine, and cysteine residues give rise to a dehydroalanine intermediate. Since such elimination reactions are second-order reactions that depend directly on the concentration of both hydroxide ions and alkali-labile amino acids, the extent of LAL formation should vary directly with hy-

Table 6—Effect of nitrogen and oxygen on lysinoalanine formation in soybean protein[a,b] (Values listed are from two separate determinations.)

Conditions	Lysinoalanine		
	Wt %	Mole %	g/16g N
65°, 1 hr, N₂	1.59; 1.72	0.85; 0.87	1.79; 1.96
65°, 1 hr, O₂	1.35; 1.45	0.77; 0.81	1.49; 1.57
65°C, 3 hr, N₂	1.91; 2.00	1.16; 1.17	2.32; 2.34
65°C, 3 hr, O₂	2.00; 2.10	1.13; 1.13	2.30; 2.40
75°C, 1 hr, N₂	1.90; 2.07	1.12; 1.15	2.25; 2.37
75°C, 1 hr, O₂	2.07; 2.16	1.11; 1.11	2.33; 2.40
75°C, 3 hr, N₂	2.45; 2.92	1.29; 1.33	3.00; 3.50
75°C, 3 hr, O₂	2.50; 3.08	1.28; 1.75	2.93; 3.81

[a] 0.5g protein in 50 mL 0.1N NaOH.
[b] Temperature in the range 65 − 75°C has only a slight effect on the solubility of O₂ or N₂ in water (Hodgman, 1958).

droxide and labile amino acid concentration. Secondly, the conjugated, reactive double bond of dehydroalanine reacts with lysine to form a LAL cross link. This step is governed by both the number and location of available ϵ-NH₂ and dehydroalanine potential partners in the protein chain. Only residues favorably situated to form crosslinks can do so. When convenient sites have reacted, additional LAL crosslinks form less readily or not at all. Each protein, therefore, may have a limited number of potential sites for forming crosslinked residues. This number is presumably dictated by the primary amino acid sequence and conformational and steric factors (Savoie and Parent, 1983).

These considerations suggest that a cascade of reactions leads to LAL formation. Thus, dehydroalanine formation may be governed not only by the absolute concentration of alkaline-labile precursors, but by their relative susceptibilities to base-catalyzed eliminations. The results of this (Tables 1, 4, 5) and previous studies (Friedman, 1978a, b) suggest that although serine and threonine destruction in soy protein, lactalbumin, and wheat gluten begins to take place near pH 12, cystine residues are much more sensitive to alkali, since most of them are destroyed between pH 9 and 10. On the other hand, the reaction of ϵ-NH₂ groups with dehydroalanine to form LAL depends not only on the cited steric and conformational factors but also on the pH of the medium, which governs the concentration of reactive, nonprotonated ϵ-NH₂ groups. Since the pK in these groups for most proteins is near 10, protonated ϵ-NH₃⁺ groups are not completely dissociated until pH reaches 12 or higher. At pH 9, for example, only about 10% of amino groups are ionized, and thus available for reaction. All of the amino groups can eventually react, however, since additional unprotonated amino groups are formed by dissociation of the protonated groups. (For mathematical analyses of relevant kinetics and equilibria, see Friedman, 1982a; Friedman and Williams, 1977.)

N-terminal α-NH₂ groups, whose pK value is near 7.5 (Friedmen et al., 1981), will interact with dehydroalanine residues to form crosslinked amino acid side chains at a pH value more than two units lower than that predicted for

Fig. 1—Possible mechanisms for (a) alkali-induced lysinoalanine formation; (b) concurrent racemization via a carbanion intermediate; and (c) basic hydrolysis via an imine intermediate (upper left-hand part of plot). See text.

ϵ-NH$_2$ groups. The potential for such α-amino group cross-linking is discussed in more detail elsewhere (Friedman and Boyd, 1977).

This mechanistic analysis suggests that the extent of LAL formation is dictated by the amino acid sequence, the number and location of unprotonated ϵ-NH$_2$ groups, the number and location of dehydroalanine precursors and their relative susceptibilities to elimination reactions, and the number and location of other protein functional groups, such as SH and α-NH$_2$ groups, which compete with lysine amino groups for dehydroalanine residues. Since a unique combination of these factors exists for each protein, the maximum amount of LAL that can be expected theoretically will vary from protein to protein. For soy protein, the observed maximum is about 40% of the original lysine content (Tables 1, 4, 5).

Since D-serine is nephrotoxic to rats (Kaltenbach et al., 1979), we have previously suggested that D-serine could potentiate the biological action of LAL (Masters and Friedman, 1979; Friedman et al., 1981). Preliminary results in Fig. 2 show that L-serine residues in soy proteins racemize rapidly to the D-isomer.

Because serine is both degraded (Tables 1, 4, 5) and racemized (Fig. 2), the cited D/L ratios may not reflect the relative content of D-serine, especially for samples treated at the higher alkalinites and temperatures, where degradation is pronounced.
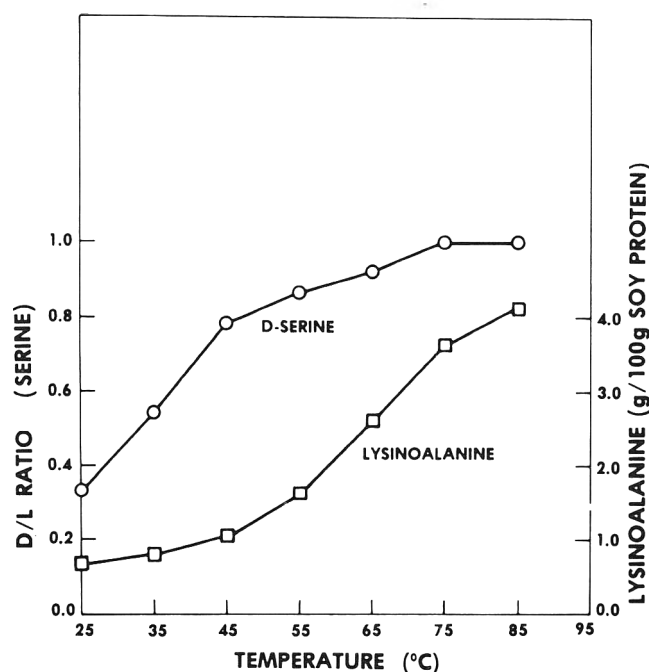
Fig. 2—Effect of temperature on the D-serine (D/L ratio) and LAL content (g/100g) content of soy proteins. Conditions: 1% protein; 0.1N NaOH; 3 hr.

Table 7—Effect of acetylation and succinylation on lysinoalanine content of alkali-treated soybean protein[a]

Treatment	Lysinoalanine (g/100g sample)		
	Unmodified protein	Acetylated protein	Succinylated protein
Untreated controls	0.00	0.00	0.00
0.1N NaOH/45°C/1 hr	0.66	0.00	0.00
0.1N NaOH/45°C/3 hr	0.92	0.00	0.00
0.1N NaOH/75°C/1 hr	1.55	0.89	0.00
0.1N NaOH/75°C/3 hr	2.18	0.72	0.00

[a] Conditions: 0.5g protein in 50 mL 0.1N NaOH.

Factors preventing lysinoalanine formation

Until the safety of LAL has been established it may be desirable to limit its formation in foods. The mechanistic pathways for LAL discussed above suggest several possible approaches to prevent or minimize LAL formation. Their applicability to soy proteins are described below.

Acylation of amino groups. Since formation of LAL requires participation of ϵ-NH$_2$ groups of lysine side chains, protection of amino groups by acetylation with acetic anhydride or succinylation with succinic anhydride should minimize LAL formation, if the protective group survives the treatment. Table 7 shows that this appears to be the case since acetylated or succinylated soy protein exposed in 0.1N NaOH at 45-75°C for 1 or 3 hr contained significantly less LAL than the untreated control. These decreases, however, were less pronounced than those observed with casein and wheat gluten (Friedman and Masters, 1982; Friedman, 1978a, b).

Effect of D-glucose. Under the influence of heat, the ϵ-NH$_2$ groups of lysine reacts with carbohydrates such as glucose to form Maillard browning products (Friedman, 1982a, b). Such reactions, if they occur in alkaline media, should reduce the formation of LAL because the blocked lysine amino groups would be unable to combine with dehydroalanine.

Table 8 shows that various glucose concentrations in soy protein suspensions exposed to 0.1N NaOH for 1 hr at 45°C did not significantly affect the amount of LAL produced when the ratio of glucose to protein ranged from 1-20 or 2-5. When the temperature of treatment was increased to 75°C, however, LAL decreases at the two concentration ratios tested (Table 9).

Tables 8-9 also show that the lysine content of the soy protein treated in the presence of glucose decreased, but not in any systematic way. In contrast, glucose seems to participate in an irreversible modification of arginine residues to derivatives stable to acid hydrolysis, as the amount of arginine present decreased systematically with increasing glucose content. Evidently, both alkaline conditions and the presence of glucose favor arginine modification. Additional studies are needed more fully to define the influence of structurally different carbohydrates on LAL formation.

The previously discussed changes also were accompanied by the appearance of unidenitified ninhydrin-positive com-

Table 8—Effect of glucose on lysine, arginine, and lysinoalanine content of alkali-treated soybean protein. Unknown compounds are designated by X [Values (g/16g N) listed are from two separate experiments]

Glucose:protein ratio	LYS	ARG	LAL	X1[e]	X2[f]
0:1	6.02	7.73	0.77	0.38	- -
	6.11	7.97	0.79	0.43	- -
1:20[a]	5.31	6.32	0.87	0.15	0.53
	5.55	6.42	0.90	0.24	0.48
1:10[b]	5.83	6.07	0.69	- -	0.65
	6.03	6.51	0.94	- -	0.30
1:5[c]	5.62	4.67	0.70	0.28	0.54
	6.29	5.48	0.80	0.42	0.46
2:5[d]	6.87	6.68	0.67	0.30	0.76
	7.13	6.85	0.67	0.46	0.53

[a] 0.5g soy protein plus 25 mg glucose in 50 mL 0.1N NaOH; 45°C; 1 hr.
[b] 0.5g soy protein plus 50 mg glucose.
[c] 0.5g soy protein plus 100 mg glucose.
[d] 0.5g protein plus 200 mg glucose.
[e] Elutes before ASP.
[f] Elutes before LAL.

pounds, designated as X in Tables 8-9. The amounts formed are related to the amount of glucose present (cf. also, Smith and Friedman, 1984). The nutritional and toxicological significance of these unknown compounds awaits further study.

Effect of metal salts. Several metal ions can interact with basic groups of amino acid residues including the guanidino group of arginine, the imidazole group of histidine, and the ϵ-amino group of lysine (Henkin, 1974). Complexation with lysine may minimize or prevent lysinoalanine formation if the complex is stable in alkali. The influence of several metal salts on the amino acid composition of alkali-treated soy protein was, therefore, examined. Table 10 shows that, within experimental error, only $CuSO_4$ and possibly $FeCl_2$ caused a significant decrease in lysinoalanine content. Histidine, arginine, and lysine were not significantly affected by the presence of any of the listed salts.

Effect of sulfhydryl compounds. Since sulfhydryl groups react more rapidly than ϵ-amino groups with dehydroalanine, addition of thiols such as cysteine or reduced glutathione should trap the residues of dehydroalanine as S-alkyl derivatives, and/or eliminate potential precursors for dehydroalanine as described below. These competitive reactions should minimize LAL formation (Friedman, 1977; Finley et al., 1978).

Table 11 shows that added thiols such as 2-aminoethanethiol, L-cysteine, N-acetyl-cysteine, reduced glutathione, and penicillamine suppress LAL formation during alkali treatment of soybean proteins. This inhibition can occur by at least three distinct mechanisms. First, by direct competition, the added nucleophile can trap dehydroalanine residues, thus inhibiting LAL formation (direct competition mechanism). Second, the added nucleophiles can cleave disulfide bonds to sulfhydryl groups. These protein SH groups will also competitively combine with dehydroalanine residues to form lanthionine (indirect competition mechanism). Third, the added nucleophile can diminish a potential source of dehydroalanine by cleaving protein disulfide bonds ($P-S-S-P$) to negatively charged sulfides ($P-S^-$). The latter will undergo elimination reactions to dehydroalanine less readily than their uncharged disulfide precursors, because the charge on $P-S^-$ will electrostatically repel negatively charged OH^- ions which initiate the eliminations (suppression of dehydroalanine formation mechanism).

Effect of dimethyl sulfoxide. Table 12 lists the LAL content of soy protein treated at $75°C$ for 1 or 3 hr in 0.1N NaOH or in 50% DMSO-50% 0.1N NaOH. For the samples treated for 1 hr in the mixed solvent, LAL concentration was about 43% less than in those exposed to the aqueous medium alone. The corresponding difference after 3 hr treatment was 52%.

This effect can be explained by reference to related previous studies (Friedman, 1967) that showed that DMSO strongly influenced the reactivities of amino groups with conjugated double-bonded compounds such as acrylonitrile. The nucleophilic addition of the $\epsilon-NH_2$ group of lysine to the double bond of dehydroalanine to form LAL is mechanistically analogous to the addition of the NH_2 groups to the double bond of acrylonitrile. Therefore, DMSO should profoundly influence the extent of LAL production. Results from an initial study (Table 12) suggest that this may indeed be the case. Additional studies are needed to more fully define the influence of nonaqueous solvents on LAL generation.

In summary, factors favoring LAL formation include high pH and temperature, and long time of exposure. Factors minimizing LAL formation include acylation of basic $\epsilon-NH_2$ groups of lysine to neutral amides and the presence of glucose, sulfhydryl compounds, copper salts, and dimethyl sulfoxide. LAL formation was accompanied by the appearance of a series of unidentified compounds whose chemical nature and nutritional and toxicological significance are presently unknown.

Table 9—Effect of glucose on lysine, arginine, and lysinoalanine content of alkali-treated soybean protein. Unknown compounds are designated by X [Values (g/16g N) listed are from two separate experiments]

Glucose:protein ratio	Apparent						
	LYS	ARG	LAL	X1[c]	X2[d]	X3[e]	X4[f]
0:1	4.18	5.53	2.04	0.59	--	--	0.86
	4.35	5.75	2.11	0.50	--	--	0.87
2:5[a]	6.00	0.66	1.21	0.30	0.77	0.99	0.41
	6.14	0.84	1.28	0.72	0.53	1.00	0.45
1:1[b]	4.65	0.71	0.80	0.22	0.55	0.72	0.42
	4.84	0.79	0.83	0.58	0.60	0.99	--

[a] Conditions: 0.5g soy protein plus 0.2g glucose in 50 mL 0.1N NaOH; 75°C; 1 hr.
[b] 0.5g soy protein plus 0.5 glucose in 50 mL 0.1N NaOH; 75°C; 1 hr.
[c] Elutes before ASP.
[d] Elutes before GLY.
[e] Elutes before LAL.
[f] Elutes before LYS.

Table 10—Effect of metal salts on lysinoalanine content of alkali-treated soy protein[a]

Metal salt treatment	Lysinoalanine			
	Weight %		Mole %	
None	1.94;	1.57[b]	1.10;	1.24
$ZnSO_4$	1.59;	1.60	0.930;	0.899
$CaCl_2$	1.88;	1.94	1.08;	1.13
$FeSO_4$	1.54;	1.82	1.00;	1.05
$FeCl_2$	1.26;	1.39	0.888;	0.901
$CuSO_4$	0.00;	0.457	0.00;	0.270

[a] Conditions: 0.5g soy protein plus 0.5 millimoles metal salt in 50 mL 0.1N NaOH; 75°C; 1 hr.
[b] Results from two separate experiments.

Table 11—Effect of thiols on lysinoalanine content of alkali-treated soy protein[a]

Additive (1 mmole)	Lysinoalanine	
	Weight %	Mole %
None	1.67	1.08
2-Aminoethanethiol	0.73	0.58
L-Cysteine	0.56	0.36
N-Acetyl-L-Cysteine	0.55	0.38
Reduced Glutathione	0.59	0.38
DL-Penicillamine	0.97	0.60

[a] Conditions: 0.5g protein in 50 mL 0.1N NaOH; 65°C; 3 hr.

Table 12—Effect of dimethyl sulfoxide (DMSO) on the lysinoalanine content of alkali-treated soybean protein[a]

Conditions	Lysinoalanine (g/16g N)
75°C/1 hr	2.39; 2.26[b]
75°C/1 hr/DMSO	1.39; 1.26
75°C/3 hr	3.44; 3.23
75°C/3 hr/DMSO	1.63; 1.57

[a] Conditions: 0.5g protein in 50 mL 0.1N NaOH or 25 mL 0.1N NaOH and 25 mL dimethyl sulfoxide (DMSO series).
[b] Results from separate experiments.

The described studies should contribute to our understanding of the chemistry and nutritional consequences of food processing. Such understanding should make it possible to devise processing conditions which minimize deleterious changes in foods.

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Effects of Heat and Ionic Strength Upon Dissociation-Association of Soybean Protein Fractions

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─────────── ABSTRACT ───────────

The effects of heat and ionic strength upon dissociation or association of soybean proteins were characterized by an immunoelectrophoresis. Among thermal products of both glycinin and β-conglycinin, the dissociated subunits of β-conglycinin retained their antigenic reactivities, while the thermal products of glycinin lost their antigenic reactivities. Appearance of immunoprecipitin arcs was dependent on the ionic strength of the solutions. This is based on the conformational changes at the ionic strengths below 0.1 where the thermal denaturation of β-conglycinin transforms from association to dissociation. The immunoelectrophoretic results can be used to determine the ionic strength of the solution in complex systems including whole soybean extracts and soybean milk. Relationship between the differences in textural properties of tofu and conformational changes of soybean protein is discussed.

INTRODUCTION

FUNCTIONAL PROPERTIES of proteins are important in food applications. Soybean protein is a highly functional and useful food protein. Minor changes in processing conditions have unexpectedly significant effects on the final food properties. There have been numerous reports on the thermal denaturation of soybean protein (Wolf and Tamura, 1969; Hermansson, 1978; Mori et al., 1982). Many investigators have proposed that the thermal denaturation of soybean protein is affected by the ionic strength of solutions (Catsimpoolas et al., 1970; Koshiyama et al., 1980-81; Hashizume et al., 1975a; Hashizume and Watanabe, 1979), and by the salt species (Iwabuchi and Shibasaki, 1981a, b). Iwabuchi and Shibasaki (1981a, b) have shown that thermal denaturation of both glycinin and β-conglycinin varies with ionic strength over a wide range from zero to 4.0. In the case of β-conglycinin, thermal transition changed from dissociation to aggregation in the presence of salt at an ionic strength near 0.1 (Iwabuchi and Shibasaki, 1981b). A conformational study revealed that heat-induced dissociates of β-conglycinin are not so unfolded but retain their secondary and tertiary structures, even after dissociation of their quaternary structures (Iwabuchi, unpublished). Accordingly, variations in these dissociation-association phenomena probably contribute to the textural properties of soybean curd. An understanding of the relationship between protein structure and its functional property should lead to an increased understanding of the molecular basis of functionality.

It is important to learn the degree of ionic strength of heat-treated soybean protein solutions, since the degree of denaturation varies with the ionic strength (Iwabuchi and Shibasaki 1981a, b). Therefore, it is expected that upon heating, proteins in soybean milk prepared by extraction with various amounts of water will exhibit different denatured states because of their different ionic strengths. Differences in their functional properties may play important roles in tofu making (Hashizume et al., 1975b).

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As previously reported, antigenic reactivities of thermally denatured products of glycinin and β-conglycinin are closely related to thermal transitions, which are affected by the ionic strength of the solutions at heating (Iwabuchi and Shibasaki 1981a, b). The objective of this paper is, therefore, to estimate the ionic strength level of soybean milk using immunological techniques.

MATERIALS & METHODS

Preparation of protein solutions

Seeds of soybean (*Glycine max* var. Raiden) were ground to a fine powder and extracted several times with 5 volumes n-hexane at room tempreature to remove lipid. The defatted meal was extracted with 5 volumes distilled water for 1 hr at room temperature. The insoluble residue was removed by centrifugation (12000 × *g*) for 30 min. The supernatant is referred to as a 1:5 meal/water extraction (Extract-5). The samples of Extract −7.5, −10, −15, and −20 were prepared by mixing 1 volume of Extract-5 with 1.5, 2, 3, and 4 volumes of water, respectively. Protein concentrations of these extracts were about 6, 4, 3, 2, and 1.5%, respectively.

Purified glycinin and β-conglycinin were prepared as described previously (Iwabuchi and Shibasaki, 1981a, b). Nonbuffered solutions of various ionic strengths were prepared by adding KCl to distilled water. Buffers of potassium phosphate (pH 7.6) were prepared as shown in Table 1.

Heat treatment

The purified protein solutions, 0.5% (w/v) protein, dissolved in various ionic strength solutions in screw-capped 16 × 125 mm test tubes, were heated in boiling water for 5 min. Water extracts of defatted meals and the diluted solutions were also heated under the same conditions.

Disc gel electrophoresis and immunoelectrophoresis

The Ornstein-Davis system gel electrophoresis with 7.5% or 6.5% separating gel and 4% stacking gel was carried out using 5 × 80 mm glass tubes. A quantity (20 µL) of each heat-treated protein solution was placed on the top of the gel. Electrophoresis was conducted for 2 hr with a constant voltage of 100V. The polyacrylamide gel column was then cut lengthwise into two parts. One part was subjected to staining with 0.3% Coomassie Brilliant Blue G 250 in methanol/acetic acid/water (45:10:45, v/v); and the other part was embedded in agar medium (1.2% agar solution in I = 0.5 phosphate buffer containing 0.4M NaCl, pH 7.6). After solidification of the agar, slots were cut parallel to the line of the column and filled with antiserum (25 µL). The reactants were allowed to diffuse at room temperature for 2 – 3 days in a chamber of high humidity. The unreacted proteins were removed by soaking the plate in 0.9% saline solution. Subsequently, the plates were stained and destained in methanol/acetic acid/water (20:7:73, v/v). The scale of the relative mobility values (Rm) was arbitrarily assigned taking the

Table 1—Formulations of potassium phosphate buffers (pH 7.6)

Buffer	Ionic strength	KH_2PO_4 (mM)	K_2HPO_4 (mM)
A	0.2	12.50	63.20
B	0.1	6.25	31.60
C	0.05	3.10	15.80
D	0.01	0.625	3.16

separating line of the stacking and separating gels as the "0.00 point," and the position of the fastest moving dye as "1.00." Results were recorded photographically.

Antisera to the native glycinin and β-conglycinin were prepared by foot pad injections into white rabbits as described previously (Iwabuchi and Shibasaki, 1981a, b).

RESULTS

Antigenicities of thermal products of glycinin and β-conglycinin

Components of thermal denaturation products of soybean protein were separated by polyacrylamide gel electrophoresis (PAGE) (Catsimpoolas et al., 1971; Hashizume et al., 1975a; Iwabuchi and Shibasaki, 1981a, 1981b). Fig. 1 shows PAGE patterns of the thermal dissociation-association products of glycinin as a function of ionic strength and their immunodiffusions against anti-glycinin serum. When glycinin was heated at ionic strengths below 0.5, many dissociated and/or associated bands appeared (Rm 0–0.68, Fig. 1A). These products, and aggregates on the top of the stacking gels, did not produce a precipitin arc with anti-glycinin (Fig. 1B). With an increase in ionic strength above 0.6, one major band (Rm 0.21) was distinctly detected, which could produce an intense precipitin arc (Fig. 1B, tube 3). This Rm 0.21 component was identical to the undissociated glycinin, which was stabilized by the presence of salt against thermal denaturation.

Thus, it is clear that the antigenicity remaining in thermally denatured products of glycinin is due to the undissociated glycinin retaining a quaternary structure. Loss of antigenicity is strongly associated with conformational changes involving dissociation into subunits, and reassociation of dissociates as reported by Catsimpoolas et al. (1971).

Fig. 2 shows the PAGE patterns of the thermal dissociation-association phenomena of β-conglycinin as a function of ionic strength. As previously reported (Iwabuchi and Shibasaki, 1981b, 1982), β-conglycinin dissociated into its subunits upon heating near zero ionic strength. However, aggregation was accelerated with slightly increased ionic strength, and dissociation bands (Rm 0.53) disappeared at the ionic strengths between 0.1 and 2.0 (Fig. 2A). Moreover, at ionic strengths above 2.0, the undissociated β-conglycinin bands appeared as a result of the stabilizing effect of salt (Fig. 2A).

Antigenicities of these thermal products of β-conglycinin were characterized by immunodiffusion against anti-β-conglycinin (Fig. 2B). Under salt-free conditions, thermal dissociates (Rm 0.53) of β-conglycinin produced a clear precipitin arc with anti-β-conglycinin. At ionic strengths between 0.1 and 2.0, aggregates on the top of the stacking gel maintained slight cross reactivity. At higher ionic strengths, both undissociated β-conglycinin and aggregates on the top of the gel produced an intense precipitin arc (Fig. 2B). The data above show that thermally denatured

A

B
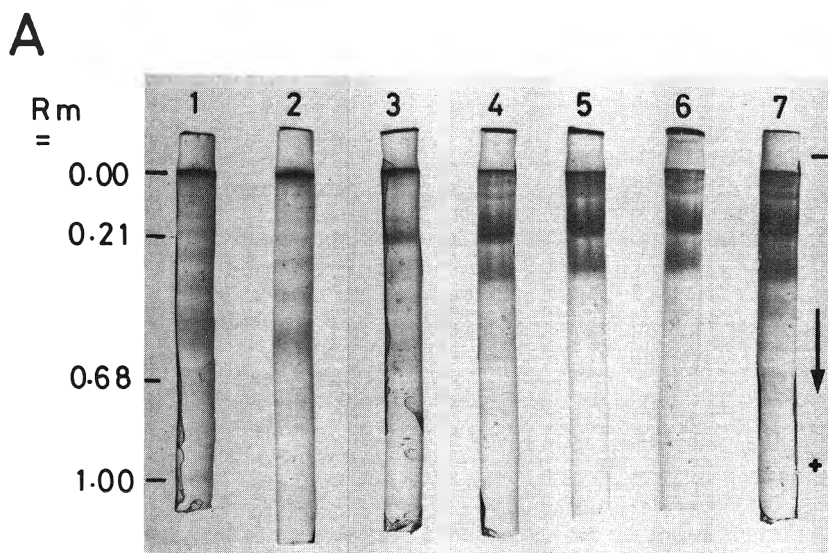
Fig. 1—(A) Disc electrophoresis patterns of glycinin solutions heated at 100°C for 5 min at various ionic strengths: (1) I = 0.1; (2) I = 0.4; (3) I = 0.6; (4) I = 0.8; (5) I = 2.0; (6) I = 2.5, and (7) unheated sample. Solutions of various ionic strengths with no buffer were prepared by adding KCl to distilled water. Electrophoresis was carried out with 7.5% separating gel and 4% stacking gel using 5 x 80 mm glass tubes. (B) Disc immunoelectrophoresis patterns, developed with anti-glycinin, of glycinin solutions heated at 100°C for 5 min at various ionic strengths. The numbers used are the same as in Fig. 1A.
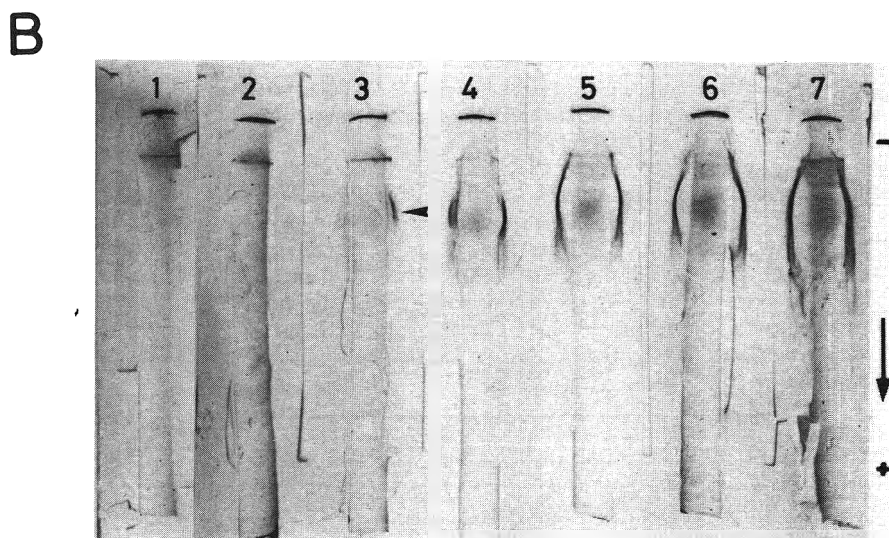
β-conglycinin did not lose its antigenic reactivity against anti-β-conglycinin; i.e., both dissociates and aggregates retained their partial cross reactivities, while thermal dissociates and aggregates of glycinin completely lost their antigenicities against anti-glycinin.

It is noteworthy that among the thermal products of both glycinin and β-conglycinin, only the thermal dissociates of β-conglycinin, except the undissociated protein molecules, are able to form a clear antigen-antibody precipitate.

Hashizume et al. (1975a, b) suggested that, from the turbidity and gel electrophoretic measurements, the ionic strength level of soybean milk, extracted with 5 – 10 volumes of water, may be below 0.2. In these ionic strength regions, no immunoprecipitin arc appeared in tubes 1 and 2 in Fig. 1B. Therefore, the information obtained by glycinin is practically unavailable as an indication of the thermal transition. On the other hand, the information of the antigenicity of heat denatured β-conglycinin is available for the determination of the ionic strength of a protein solution. Furthermore, it is necessary to precisely study the regions below $I = 0.1$ where the thermal transition of β-conglycinin changes from association to dissociation.

Fig. 3 shows the thermal transitions of β-conglycinin at low ionic strengths. The effect of a salt species on the thermal dissociation-association behavior varied depending on whether the salt was KCl or potassium phosphate.

That is, in the case of potassium phosphate, thermal dissociates (Rm 0.55) disappeared above $I = 0.1$ but the undissociated and/or reassociated component (Rm 0.08) remained even in the presence of $I = 0.1$ (Fig. 3A, tube 2). In the case of KCl, thermal dissociates (Rm 0.55) disappeared in the presence of $I = 0.05$ and the Rm 0.08 component disappeared at $I = 0.1$ (Fig. 3A, tube 5). Thus, it is clear that the two types of salts show quite different behavior near $I = 0.05 - 0.1$.

A clear immunoprecipitin arc was observed in tubes 4 and 8 in Fig. 3B. The appearance of an immunoprecipitin arc is closely related to the ionic strength of the protein solution, i.e., the extent of dilution. Therefore, when the soybean extracts are prepared, the ionic strength can be estimated from the extent of dilution. This is based on the facts that the thermal dissociates of β-conglycinin retain their antigenic activities against anti-β-conglycinin and the formation of thermal dissociates is exactly related to the ionic strength of the solutions containing β-conglycinin.

Characterization of water extracts of defatted soybean meals as a function of dilution ranges

The results above were applied in estimating the ionic strength levels of water extracts of defatted soybean meals. Using water extracts of meals (Extract-5, −7.5, −10, −15, and −20) with various volumes of water, measurements of PAGE and immunological reactivity were
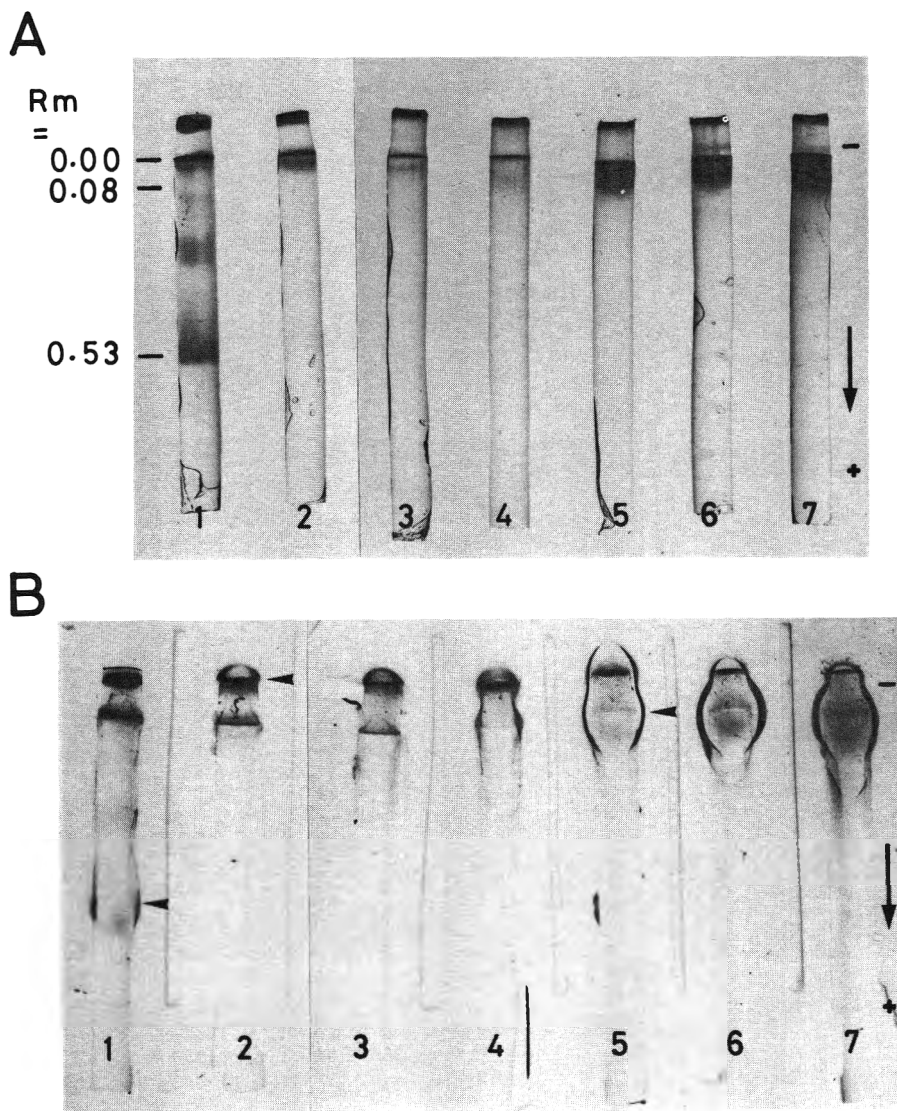
Fig. 2—(A) Disc electrophoresis patterns of β-conglycinin solutions heated at 100°C for 5 min at various ionic strengths: (1) I = 0; (2) I = 0.2; (3) I = 1.5; (4) I = 2.0; (5) I = 2.25; (6) I = 3.0, and (7) unheated sample. Solutions of various ionic strengths with no buffer were prepared by adding KCl to distilled with no buffer were prepared by adding KCl to distilled water. Electrophoresis was carried out with 7.5% separating gel. (B) Disc immunoelectrophoresis patterns, developed with anti-β-conglycinin, of β-conglycinin solutions heated at 100°C for 5 min at various ionic strengths. The numbers used are the same as in Fig. 2A.

carried out (Fig. 4). There were no distinct differences in the PAGE patterns, qualitatively, although some components (e.g. Rm 0.55 component) quantitatively increased with an increase in the dilution ranges. Moreover, PAGE patterns were complicated by the presence of thermal products of many proteins such as glycinin, hemagglutinin, lipoxygenase, etc. Therefore, it is inadequate to use PAGE patterns for the purpose of this study.

On the other hand, immunological reactivities between whole soybean proteins and anti-β-conglycinin were not affected by the presence of other proteins. Immunoelectrophoresis, therefore, is an excellent tool to estimate the ionic strength of the water extracts of soybean meals. The increase in dilution ranges was accompanied by a distinct appearance of an immunoprecipitin arc between the thermal dissociates of β-conglycinin and anti-β-conglycinin. A characteristic transition from aggregation to dissociation was observed between Extract-10 and Extract-15 (Fig. 4B). Comparing Fig. 4B with Fig. 3B, the effects of ionic strength on the thermal dissociation-association of water

extracts of defatted soybean meals, which were extracted with 5, 7.5, and 15 volumes of water, were equal to those of I = 0.15, 0.1, and 0.05 in Fig. 3B, respectively. The salt type of water extracts was considered to be potassium phosphate rather than KCl since the immunological behavior of the component of Rm 0.08 (Fig. 4B, tube 3), one of major thermal products originating from β-conglycinin, was similar to that of the potassium phosphate series shown in Fig. 3B (tube 2). This concept may be supported by the mineral composition of soybeans (Smith and Circle, 1972). Measurements of ionic strength were uncorrected for the effect of non-salt components which are contained in whole water extracts and probably have an effect on the thermal denaturation of soybean protein.

Thermal dissociation-association of glycinin is affected by the protein's concentration (Mori et al., 1982). However, in the case of β-conglycinin, thermal dissociation-association behavior was affected more by the change of ionic strength than by that of concentration caused by dilution (data not shown).
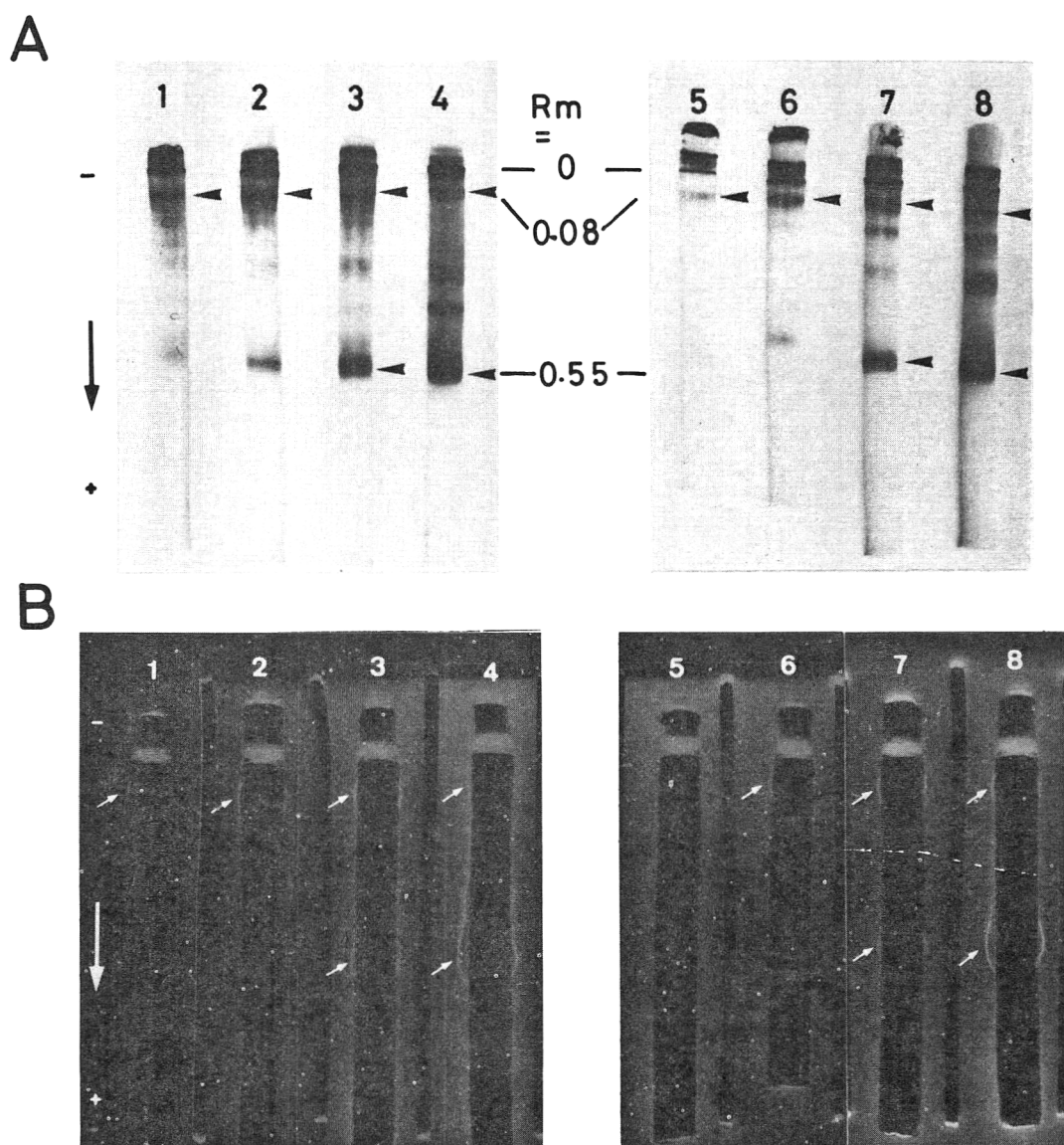
Fig. 3—Thermal transitions of β-conglycinin from aggregation to dissociation under conditions of low ionic strengths. (A) Disc electrophoresis patterns of β-conglycinin solutions heated at 100°C for 5 min at various ionic strengths: (1) I = 0.2; (2) I = 0.1; (3) I = 0.05, and (4) I = 0.01 in potassium phosphate solutions, and (5) I = 0.1; (6) I = 0.05; (7) I = 0.025, and (8) I = 0.01 in KCl solutions. Electrophoresis was carried out with 6.5% separating gel. (B) Disc immunoelectrophoresis patterns, developed with anti-β-conglycinin, of β-conglycinin solutions heated at 100°C for 5 min at various ionic strengths. The numbers used are the same as in Fig. 3A.

DISCUSSION

THE ENTIRE ARGUMENT of this paper is that the differences observed in the immunoelectrophoretic patterns, that relate to heat and ionic strength effects upon dissociation or association of soybean proteins, can be used to determine the ionic strength of a solution. We tried to use these results to speculate the relationship between the differences in textural properties of tofu and changes in the thermally denatured states of soybean proteins.

The data presented indicate that the components of the thermal products of soybean meal extracts, prepared by adding 5 – 10 and 15 – 20 volumes of water, should vary and are probably composed mainly of aggregates and dissociates, respectively. Unless otherwise noted, the discussion concerning the thermal dissociation-association of whole soybean proteins is limited to that of β-conglycinin. With glycinin, it appears that heat-induced dissociates of glycinin were transformed to their aggregates at the extract's ionic strength approximately I = 0.1 (Hashizume et al., 1975a; Iwabuchi and Shibasaki, 1981a).

The information obtained from the water extracts of defatted soybean meals may be able to apply to understand soybean milk as pointed out by Hashizume (1975a, b).

As is known, soybean curd (tofu) is made by coagulating soybean milk with calcium salts or glucono-delta-lactone.

In Japan, there are various types of tofu such as Kinu, Soft, Momen, and fresh tofu for Kori tofu which are prepared with soybean milk of 1:5, 1:7, 1:10, and 1:15 soybean/water extractions, respectively. The textural properties of these tofus have minute variations and have a tendency to become harder with increasing volumes of water (Hashizume et al., 1975b; Saio, 1978, Saio and Watanabe, 1978).

Saio (1978) has suggested the relationship between a fine structure by scanning electron microscopy and texture of tofu as follows: The density of the network of tofu, which varies with the concentrations of soybean milk and coagulant, correlates with the hardness of tofu; the larger the protein granules aggregated with calcium salt in the network are, such as Kori tofu made by more dilute concentration of soybean milk, the harder the tofu. This type of Ca-coagulate may easily lose the capacity of water folding in gel networks and becomes hard tofu. On the other hand, the network density of Kinu or Soft tofu is coarse (Saio, 1978). This may cause the difficulty in releasing water from the Ca-coagulates and results in soft tofu.

Characteristics of tofu may be influenced by the changes of the thermally denatured states of β-conglycinin in soybean milk. Using the above mentioned principle of tofu and the results in Fig. 4, we tried to interpret the textural prop-
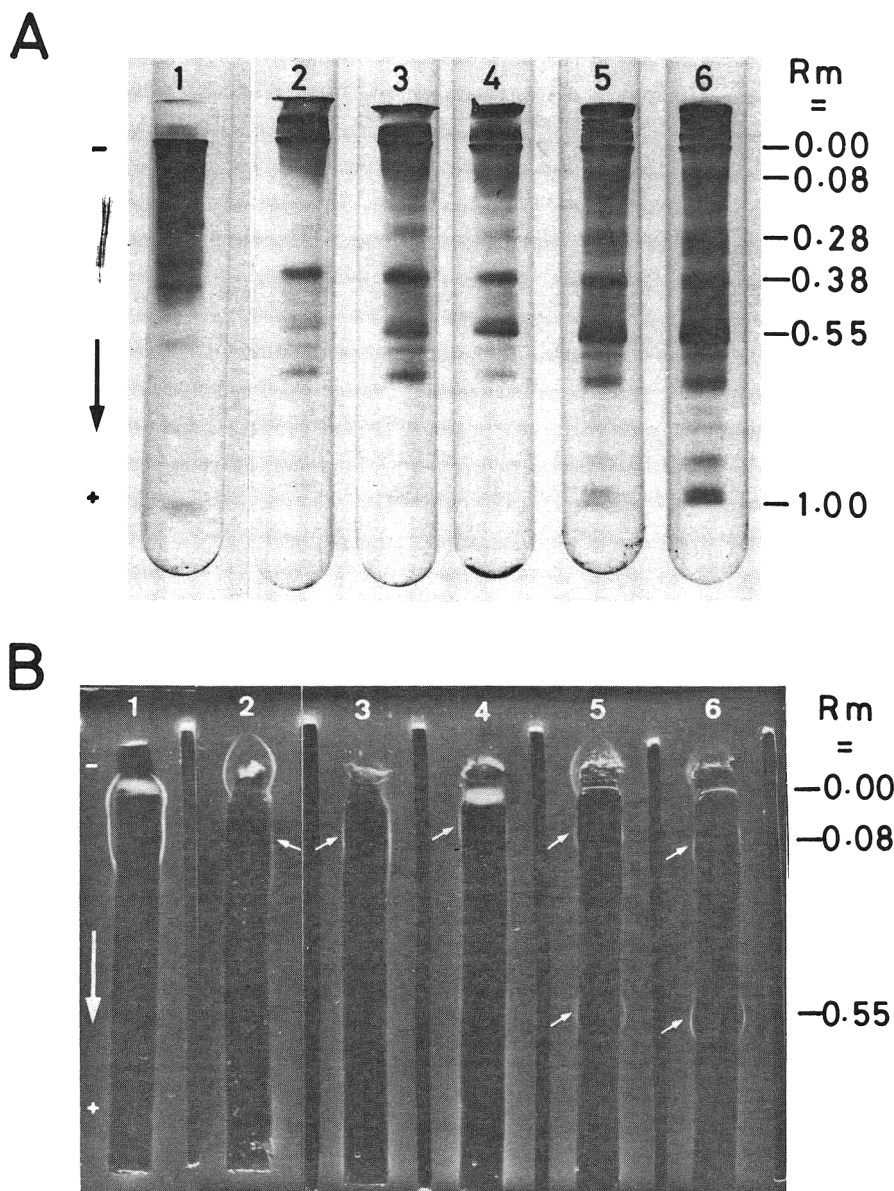
Fig. 4—(A) Disc electrophoresis patterns of whole water extracts of defatted soybean meals heated at 100°C for 5 min: (1) unheated extract; (2) Extract-5; (3) Extract-7.5; (4) Extract-10; (5) Extract-15, and (6) Extract-20. Electrophoresis was carried out with 6.5% separating gel. (B) Disc immunoelectrophoresis patterns, developed with anti-β-conglycinin, of whole water extracts of defatted soybean meals heated at 100°C for 5 min as a function of dilution ranges. The numbers used are the same as in Fig. 4A.

erties of tofu from the dissociation-association viewpoint of the thermal denaturation of β-conglycinin as follows: When soybean milks prepared with low volumes of water (5 – 7 volumes) are heat-treated, heat-induced dissociates of β-conglycinin will soon aggregate since this soybean milk is believed to contain I = 0.1 – 0.2 salts (Fig. 4B). These thermally denatured states of soybean milk may correlate with the textural properties of soft tofu after calcium coagulation. On the other hand, when soybean milk is prepared with more water, above 15 volumes, and heated, a large amount of thermal dissociates of β-conglycinin will remain in the soybean milk since the ionic strength of soybean milk is believed to be below 0.05. The thermally denatured states, under these ionic strengths, may correlate with the hard tofu after calcium coagulation.

The results of the immunological behaviors (Fig. 1 and 2), that is, both the thermal dissociates and aggregates of β-conglycinin, still had a distinct and/or partial cross reactivity against anti-β-conglycinin while those from glycinin did not. This suggests that since the antigenic determinants of glycinin are probably conformation dependent, glycinin completely loses its antigenicity when the quarternary structure of the glycinin molecule is disrupted. On the other hand, the antigenic determinants of β-conglycinin are probably located on each subunit of α, α', and β.

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and El Sahrigi, 1964; Triebes and King, 1966). Thus from Eq. A6:

$$K \cong 0.02 \text{ for frozen meat.}$$

Also, r/s < 1.0 (Fig. 2).

Hence, for frozen meat, from Eq. A10, the maximum relative error in ignoring the term $(K \, k_y R_m)$ or $(h_c R_h)$ is only 0.02, or 2%. In the usual frozen storage temperature range ($-10°C$ or less) the error is even less (<1%). For other biological products the errors will be of the same order, since k_s and D_s are of similar magnitude (Harper and El-Sahrigi, 1964; Triebes and King, 1966; Harper, 1962; Sandall et al., 1967).

Thus, Eq. (14) in the text is a good approximation to Eq. (13).

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Kinetic Energy Calculations for Non-Newtonian Fluids in Circular Tubes

FERNANDO A. OSORIO and JAMES F. STEFFE

——————————ABSTRACT——————————

Expressions to calculate the kinetic energy of Herschel-Bulkley fluids flowing in circular tubes are presented. The influence of yield stress, consistency coefficient and flow behavior index are investigated. An equation — based on theoretical considerations — for determining kinetic energy from a kinetic energy correction factor is developed. Solutions are given in a graphical format to facilitate kinetic energy calculations.

INTRODUCTION

Literature review

Solving design problems related to non-Newtonian fluids flowing in circular tubes requires a knowledge of the energy requirements related to the changes in kinetic energy. An expression for kinetic energy is generally presented as a separate term in the mechanical energy balance equation (Heldman and Singh, 1981). The average kinetic energy per unit mass (KE) of any fluid stream moving in a round pipe is (Skelland, 1967):

$$KE = \frac{1}{R^2 \bar{u}} \int_o^R r\, u^3\, dr \qquad (1)$$

Eq. (1) has been solved for the laminar flow of Newtonian fluids (Geankoplis, 1978), power-law fluids (Metzner, 1956), Bingham plastic fluids (McMillen, 1948; Metzner, 1956). In addition, Steffe and Osorio (1983) solved Eq. (1) for the laminar flow of Herschel-Bulkley (H-B) fluids; the current work is an advancement and expansion of that effort.

The KE in laminar flow can be expressed in terms of a kinetic energy correction factor as

$$KE = \frac{\bar{u}^2}{\alpha} \qquad (2)$$

where α is the kinetic energy correction factor. For Newtonian fluids $\alpha = 1$ and for power-law fluids (Metzner, 1956),

$$\alpha = \frac{(4n+2)\,(5n+3)}{3\,(3n+1)^2} \qquad (3)$$

To date, an analytical expression for the kinetic energy correction factor of a H-B fluid has not been available.

For the case of turbulent or plug flow the velocity profile is negligible and Eq. (1) may be simplified to

$$KE = \frac{(\bar{u})^2}{2} \qquad (4)$$

The ratio of actual KE [Eq. (2)] to the turbulent or plug flow KE [Eq. (4)] has been solved for Bingham plastic fluids (McMillen, 1948). A similar expression for a H-B fluid has not been available.

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Objectives

The objectives of this work were: (1) to obtain an analytical expression for the kinetic energy correction factor, α, of a H-B fluid; (2) to obtain an analytical expression for the ratio of the actual kinetic energy to the kinetic energy in the plug flow (KE actual/KE plug) of a H-B fluid flowing under laminar conditions in a circular tube; and (3) to present the above solutions in graphical form.

The kinetic energy correction factor (α) and kinetic energy ratio (KE actual/KE plug) were selected as parameters because they result in expressions which can be easily used in solving food engineering problems.

THEORETICAL DEVELOPMENT

THE H-B MODEL, which accurately describes the flow behavior of many fluid foods, is expressed as (Herschel and Bulkley, 1926):

$$\tau = k\,(\dot{\gamma})^n + \tau_o \qquad (5)$$

This is a generalized form of two frequently used flow models

$$\tau = k\,(\dot{\gamma})^n \qquad (6)$$

$$\tau = k\,(\dot{\gamma}) + \tau_o \qquad (7)$$

where Eq. (6) and (7) refer to the power-law and Bingham plastic models, respectively.

For the steady-state flow of an incompressible fluid under isothermal conditions, the mechanical energy balance is commonly written as (Heldman and Singh, 1981):

$$Z_1 + \frac{P_1}{\rho} + (KE)_1 + W = Z_2 + \frac{P_2}{\rho} + (KE)_2 + E_f \qquad (8)$$

where the subscripts indicate points 1 and 2 in the fluid handling system. Recall, KE is the average kinetic energy per unit mass; hence, KE must be evaluated to effectively use Eq. (8).

Considering a force balance on the fluid in a circular tube of length L and radius R, the force impelling the element of fluid to move downstream is $\delta P \pi r^2$, and the force retarding this movement is $\tau 2\pi r L$. Since the fluid is flowing with a constant velocity, the net forces acting on the cylinder of fluid must be zero. Then, equating the above expressions and solving for shear stress provides

$$\tau = \frac{\delta P r}{2L} \qquad (9)$$

Substituting Eq. (5) into this expression and using the fact that the rate of shear, $\dot{\gamma}$, is equal to $-du/dr$, gives

$$\frac{-du}{dr} = \frac{1}{k^{1/n}} \left[\frac{\delta P r}{2L} - \tau_o \right]^{1/n} \qquad (10)$$

Eq. (10) yields two expressions which completely describe the velocity profile as (Charm, 1978):

$$u = \left(\frac{2L}{\delta P k^{1/n}} \right) \left(\frac{1}{\frac{1}{n}+1} \right) \left[\left(\frac{\delta P R}{2L} - \tau_o \right)^{\left(\frac{1}{n}+1\right)} - \left(\frac{\delta P r}{2L} - \tau_o \right)^{\left(\frac{1}{n}+1\right)} \right]$$

$$\text{for } \frac{\tau_o 2L}{\delta P} < r \leqslant R \qquad (11)$$

and

$$u = \left(\frac{2L}{\delta P k^{1/n}} \right) \left(\frac{1}{\frac{1}{n}+1} \right) \left[\left(\frac{\delta P R}{2L} - \tau_o \right)^{\left(\frac{1}{n}+1\right)} \right]$$

$$\text{for } 0 \leqslant r \leqslant \frac{\tau_o 2L}{\delta P} \qquad (12)$$

The total volumetric rate of flow in a tube may be determined by considering the flow rate in two parts: first, in that region where the yield stress is exceeded; and second, where the yield stress has not been exceeded (Charm, 1978). This consideration results in

$$Q = \int_{\frac{\tau_0 2L}{\delta P}}^{R} u 2\pi r \, dr + \int_{0}^{\frac{\tau_0 2L}{\delta P}} u 2\pi r \, dr = \bar{u} \pi R^2 \qquad (13)$$

From Eq. (13), the mass average velocity $\bar{u}$ was evaluated, and can be written as:

$$\bar{u} = \left[\frac{\delta PR}{2Lk}\right]^{1/n} R n \left\{ \frac{(1-C)^{\left(\frac{1}{n}+1\right)}}{(1+n)(1+2n)(1+3n)} \right\}$$
$$\left[1+3n+2n^2+2n^2C^2+2nC+2n^2C^2\right] \qquad (14)$$

where $C = \tau_0/\tau_w = \tau_0/(\delta PR/2L)$.

By using Eq. (11) and (12), Steffe and Osorio (1983) evaluated the integral in Eq. (1) as, in a rearranged form,

$$\frac{KE}{\left(\frac{\delta PR}{2Lk}\right)^{2/n} R^2} = \frac{n^2 (1-C)^{\left(\frac{2}{n}+2\right)}}{2 (1+n)^2} \left[\frac{1}{(1+3n+2n^2+2n^2C^2+2nC+2n^2C^2)}\right]$$
$$\left[\frac{18+n(105+66C)+n^2(243+306C+85C^2)+n^3(279+522C+350C^2)}{(2+3n)(3+5n)(3+4n)}\right.$$
$$\left.+ \frac{n^4(159+390C+477C^2)+n^5(36+108C+216C^2)}{(2+3n)(3+5n)(3+4n)}\right] \qquad (15)$$

KE, calculated in terms of mass average velocity, $\bar{u}$, is obtained by combining Eq. (14) and Eq. (15) giving,

$$KE = \frac{\bar{u}^2 (1+2n)^2 (1+3n)^2}{2(1+3n+2n^2+2n^2C+2nC+2n^2C^2)^3}$$
$$\left[\frac{18+n(105+66C)+n^2(243+306C+85C^2)}{(2+3n)(3+5n)(3+4n)}\right.$$
$$\left.+ \frac{n^3(279+522C+350C^2)+n^4(159+390C+477C^2)+n^5(36+108C+216C^2)}{(2+3n)(3+5n)(3+4n)}\right] \qquad (16)$$

Dividing Eq. (16) by Eq. (4), the ratio of the actual kinetic energy (KE actual) to that calculated by assuming plug flow (KE plug) is

$$\frac{KE \text{ actual}}{KE \text{ plug}} = \frac{(1+2n)^2 (1+3n)^2}{(1+3n+2n^2+2n^2C+2nC+2n^2C^2)^3}$$
$$\left[\frac{18+n(105+66C)+n^2(243+306C+85C^2)}{(2+3n)(3+5n)(3+4n)}\right.$$
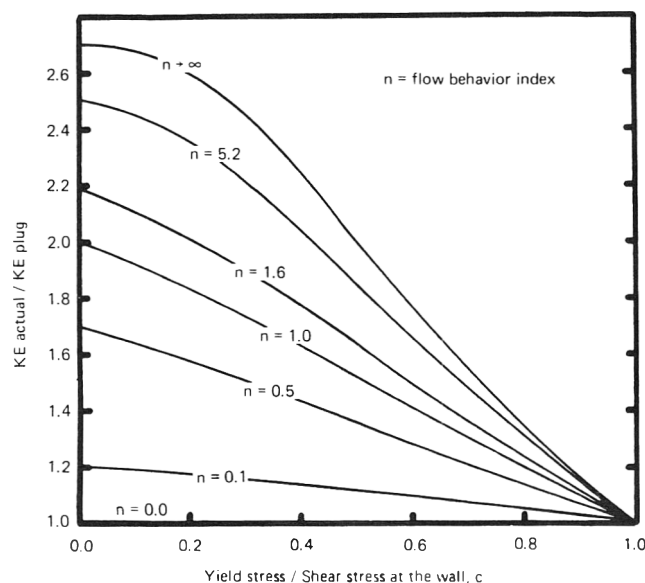
$$\left.+ \frac{n^3 (279+522C+350C^2)+n^4 (159+390C+477C^2)+n^5 (36+108C+216C^2)}{(2+3n)(3+5n)(3+4n)}\right] \qquad (17)$$

By combining Eq. (14) and Eq. (15) and by equating this expression to Eq. (2), a kinetic energy correction factor, α, is obtained for H-B fluids as,

$$\alpha = [2(1+3n+2n^2+2n^2C+2nC+2n^2C^2)^3 (2+3n)(3+5n)(3+4n)]/$$
$$\left[(1+2n)^2 (1+3n)^2 \right] \left[18+n(105+66C)+n^2 (243+306C+85C^2) \right.$$
$$+ n^3 (279+522C+350C^2) + n^4 (159+390C+477C^2)$$
$$\left. + n^5 (36+108C+216C^2) \right] \qquad (18)$$

GRAPHICAL PRESENTATION OF RESULTS

VALUES of the KE actual/KE plug are plotted in Fig. 1 for the entire C range, with n values as parameters varying from 0.0 to $n \to \infty$. The KE of the H-B fluid in laminar flow is close to the value found for a fluid in turbulent flow when C is large. The maximum KE ratio is 2.7 when n is infinite and C is zero. When n = 1.0 (Bingham plastic model) the values are the same as those obtained by McMillen (1948). The KE of the Herschel-Bulkley fluid, when n approaches zero, is about the same as that found for a fluid in turbulent flow. When C = 0.0 and n = 1.0 (Newtonian fluid) the ratio is 2.0. Note that the KE ratio is most sensitive to the flow behavior index when C is small.

Values of the kinetic energy correction factor for H-B fluids [Eq. (18)] are plotted in Fig. 2 for the entire C range with n values varying from 0.0 to $n \to \infty$. The α value of the H-B fluid is about the same as the value for turbulent flow when C is large. When n = 1.0 and C = 0.0 (Newtonian fluid) the α value is 1.0. The minimum α value is 0.74 for C = 0.0 and $n \to \infty$. The α value of a Herschel-Bulkley fluid in laminar flow with n approaching zero is the same value as that found for a fluid in turbulent flow. When C = 0.0 (power-law fluids) the values are the same as those reported by Metzner (1956).

If the parameters k, n, τ_0 and C related to a H-B fluid are known, the average kinetic energy per unit mass — to be used in Eq. (8) — can be estimated by using Fig. 2 to obtain the kinetic energy correction factor. By using this value of α in Eq. (2), KE for laminar flow is obtained. The mass average velocity, $\bar{u}$, can be calculated from the total volumetric flow rate.

Fig. 1—Kinetic energy ratio versus τ_0/τ_w at various values of the flow behavior index.
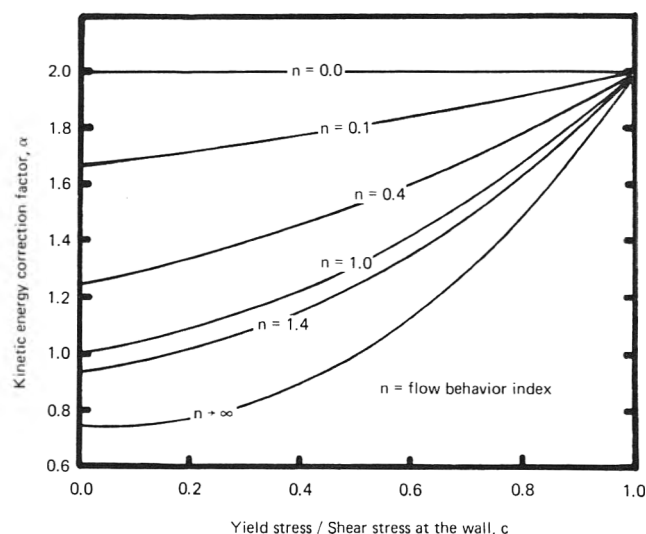
Fig. 2—Kinetic energy correction factor for Herschel-Bulkley fluids versus τ_0/τ_w at various values of the flow behavior index.

Characterization of Zinc Components on in vitro Enzymatic Digestion of Foods

SAYOKO IKEDA

─────ABSTRACT─────

Beef and polished rice were subjected to in vitro digestion and the soluble zinc components separated by gel filtration. Approximately 30% of beef zinc was solubilized by digestion; and more than 30% of rice zinc was solubilized. Most of the soluble zinc was found in one or two proteinaceous components with molecular weight of 1,270–5,130 dalton. Amino acid analyses showed that these proteinaceous components were high in the acidic amino acids and glycine, and low in L-histidine. These findings suggest that these proteinaceous components, as they combine with zinc, may increase the amount of soluble zinc.

INTRODUCTION

ZINC is an essential micronutrient for humans. Nutritional deficiency of zinc is fairly prevalent throughout the world and has been one of the major nutritional problems (Prasad, 1966; Sandstead, 1973; Moynahan and Barnes, 1973). A recommended dietary allowance for zinc has been established in several countries during the past decade (IUNS, 1982).

Evaluation of diets for zinc adequacy requires knowledge of both the amount and the availability of the zinc present. While information on the zinc content of foods is reasonably adequate (Murphy et al., 1975; Freeland and Cousins, 1976), knowledge of food zinc availability is incomplete. In recent years, many researchers have attempted to clarify the mechanism responsible for the intestinal absorption of zinc. Considerable attention has been directed toward the identification of zinc binding ligands which may enhance the intestinal absorption of ingested zinc (Evans and Johnson, 1980; Lönnerdal et al., 1980; Song and Adham, 1978; Richards and Cousins, 1975). At present, there is no consensus on the mechanism of zinc absorption.

Zinc availability has been found to be affected by several dietary factors, which include proteins, calcium, tin, dietary fiber and phytic acid (O'Dell, 1969; Johnson et al., 1982; Reinhold et al., 1976). These findings suggest that the chemical form in which zinc is presented to the absorptive cells may have a profound influence on the availability of ingested zinc. However, the chemical form in which dietary zinc occurs in the gastrointestinal tract remains uncertain. In addition, it is largely unknown whether or not the intestinal absorption of zinc is facilitated by naturally occurring ligands, such as peptides, organic acids and oligosaccharides, which originate in the gastrointestinal intermediate metabolism of major dietary constituents including proteins and carbohydrates. Thus, information about the chemical form of dietary zinc in the alimentary tract is needed in order to evaluate the exact availability of food zinc. This study was undertaken to clarify the distribution of zinc on the in vitro enzymatic digestion of foods, and to characterize the zinc binding components present in the foods subjected to in vitro digestion.

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MATERIALS & METHODS

Materials

Two different samples consisting of animal and plant food products as sources of zinc were selected for this investigation: beef and polished rice. Polished white rice from a commercial mill, and fresh ground beef were purchased locally and used immediately. Prior to analysis, polished rice was cooked as usually prepared: the raw rice (about 150g) was washed well with distilled water to remove all traces of bran and then immersed in 1.5-fold (V/W) volume distilled water for about 1 hr. It was cooked for about 20 min in a 1-liter electric rice cooker, and then left covered in the cooker for about 15 min to attain optimal eating quality. Beef was heated at 95°C in five-fold (V/W) volume distilled water for 5 min. The zinc contents of the fresh ground beef and the cooked rice were 4.01 ± 0.13 mg and 0.42 ± 0.08 mg/100g fresh weight (means $\pm$ S.D.), respectively. The ground beef and the cooked, polished rice contained 17.3% and 2.6% protein on a fresh weight basis, respectively. Pepsin (from hog stomach mucosa, 2 × cryst., 1:60,000) was obtained from Sigma Chemicals Co.; and pancreatin NF from Difco Laboratories. Dialysis membrane (Cello-tube VT 351), which was able to cut off passage of materials with molecular weight of 3,500 dalton or over, was a product of Visking Co.

In vitro proteolytic digestion

All determinations were carried out five times on four to six different samples of each food. Proteolytic digestion was performed according to the method of Akeson and Stahmann (1964) with a slight modification (Ikeda, 1982). Peptic digestion was performed in 0.06N hydrochloric acid for 3 hr at 37°C. The enzyme-to-protein ratio was 1:100. Immediately after peptic digestion, the incubates were adjusted to pH 8.0 with 2M Tris-HCl buffer. A pancreatin solution was then added to the digestion mixtures (enzyme-to-protein ratio was 1:20) and incubated for an additional 20 hr at 37°C. Sodium azide was added to the digestion medium to a final concentration of 0.025% to prevent growth of microorganisms. Immediately after digestion, the suspensions were placed in an ice-cold vessel to diminish enzymatic action, and then clarified by centrifugation (10,000 × g, 15 min). The supernatants were concentrated in a freeze-drier to approximately half of the original volume and subsequently applied on a Sephadex G-50 column (1.6 × 90 cm), which had been pre-equilibrated against 0.1M Tris-HCl buffer (pH 8.0). Gel filtration chromatography has been shown to be effective in the separation of zinc-binding components in foods (Evans and Johnson, 1980; Eckhert et al., 1977). Most of zinc components present in the food digesta were recovered as one or two peak fraction(s), and were designated as the zinc-fractions. Gel filtration chromatography was performed three times on four different samples of each food; and the elution profiles reported in the figures were reproducible in all the chromatographic analyses done.

Recovery of zinc in the in vitro digestion procedure was determined by adding five-fold molar amounts of zinc sulfate, based on the concentration of endogenous zinc, to the digestion mixtures of beef and polished rice, respectively. After digestion, approximately 97% of the exogenous zinc added was recovered.

Amino acid analyses

Protein samples were hydrolyzed with 6N hydrochloric acid for 22 hr at 110°C in evacuated, sealed tubes. Amino acid analyses were performed on a Yanaco LC-8 amino acid analyzer.

Zinc-relative affinity measurements

The relative affinity of the zinc-fractions for zinc was estimated by the equilibrium dialysis procedure (Klotz et al., 1946). One

milliliter aliquots of the zinc-fractions (0.25-0.73 μg zinc/mL), obtained by gel filtration procedure on Sephadex G-50 as described above, were dialyzed for 20 hr either against 300 mL of 0.1M Tris-HCl buffer (pH 7.2), or against 300 mL of the same buffer containing 0.1 or 1 mM of several chelating compounds. The dialyzable portions were subsequently assayed for zinc.

Zinc-binding capacity measurements

The binding capacity of the zinc-fractions for zinc was determined by an analytical gel filtration chromatographic procedure (Hummel and Dreyer, 1962). Attempts were first performed to remove the endogenous zinc from the zinc-fractions. The zinc-fractions were incubated with an excess of ethylenediaminetetraacetic acid (EDTA) for 2 hr at room temperature. After incubation, the mixtures were applied to a Sephadex G-10 column (2.0 × 40 cm) using 0.1M Tris-HCl buffer (pH 7.2) as eluant. This chromatographic procedure resolved the mixture into the entirely zinc-free fractions and EDTA-Zn^{2+} complex. The entirely zinc-free fractions were pooled and used in zinc-binding studies. Aliquots of the entirely zinc-free fractions were applied to a Sephadex G-10 column (1.0 × 37 cm), pre-equilibrated with 0.2M Tris-HCl buffer (pH 7.2) containing 10 μM $ZnSO_4 \cdot 7H_2O$. The column was then eluted with this Zn^{2+}-containing buffer solution at 45 mL/hr. The zinc concentration in column effluents was then determined. The zinc-binding capacity was estimated from the area of the trough at the elution profiles obtained (Hummel and Dreyer, 1962).

Other analyses

The distribution of protein in column effluents was determined by A_{280} measurements. Protein content was estimated by the micro-Kjeldahl method (N × 6.25) (AOAC, 1980), and peptide content according to either the method using 2,4,6-trinitrobenzene-sulfonic acid (TNBS) (Goldfarb, 1966) or the procedure of Lowry

et al. (1951). Carbohydrate content was estimated using the phenol-sulfuric acid procedure (Dubois et al., 1956). The zinc content of samples was determined with a Hitachi 208 atomic absorption spectrophotometer. In determining zinc in solid food samples, the samples were wet-ashed with sulfuric acid and 30% hydrogen peroxide prior to atomic absorption spectrophotometry. Phosphorus was assayed according to the method of Bartlett (1959).

Disc gel electrophoresis was carried out in 7.5% polyacrylamide gel at pH 8.6 by the procedure of Davis (1964). The gel was stained for protein with Amido Black 10B and then destained by washing with 7% acetic acid. Densitometric scanning of gels was done with an Ozumar 802 densitometer. In order to detect zinc in the gel, the stained gel was horizontally sliced in approximately 3 mm thick slices. Gel slices were crushed in 5 mL 10% trichloroacetic acid. After elution for 1 hr at room temperature, the crushed gels were removed by centrifugation, and the supernatant solutions were assayed for zinc.

RESULTS

Chromatographic behavior of zinc

The soluble zinc in the digesta of beef constituted 28.3 ± 2.4% (means ± S.D.; n=4) of the total zinc of beef (Fig. 1). Gel filtration chromatography of the beef digesta resulted in the appearance of a single peak containing zinc. The recovery of zinc on chromatography was 95% or over. A significant amount of peptides, as determined by the TNBS procedure, was detected in this peak fraction (Fig. 1b). This fraction showed a typical protein UV absorption pattern; its pattern had a maximum at 278 nm ($E_{1 cm}^{1\%}$=6.24) and minimum at 250 nm ($E_{1 cm}^{1\%}$=3.66) with a discernible shoulder at 288 nm (data not shown). In addition, phosphorus emerged together with zinc (Fig. 1). These findings indicate
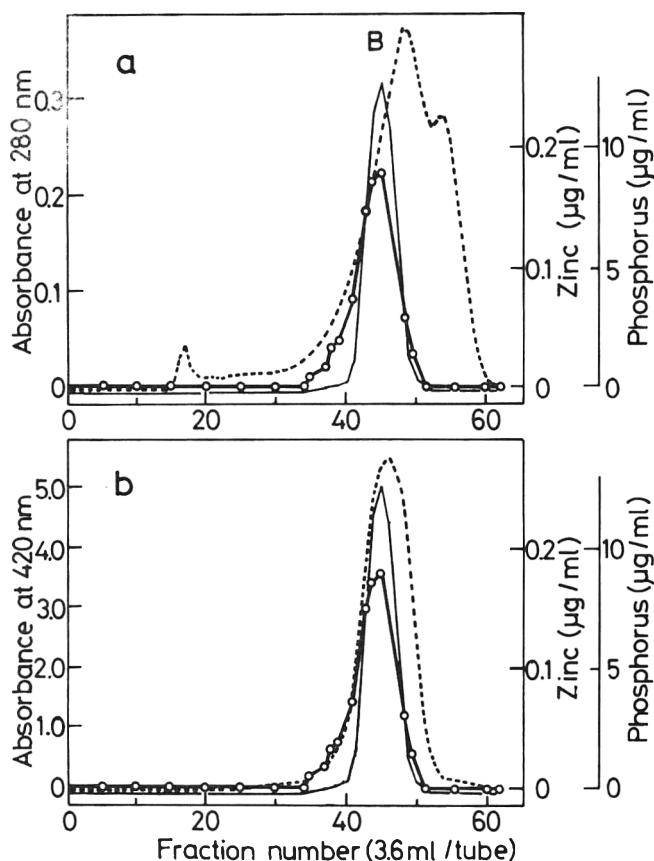
Fig. 1—Gel filtration patterns of the soluble zinc-fraction occurring from the in vitro digestion of beef meat. The zinc peak fraction obtained by the chromatographic procedure was designated as the fraction B. ——○——, zinc; ———, phosphorus; ·······, absorbance at 280 nm (in a); and ·······, absorbance at 420 nm after incubation with TNBS (in b).
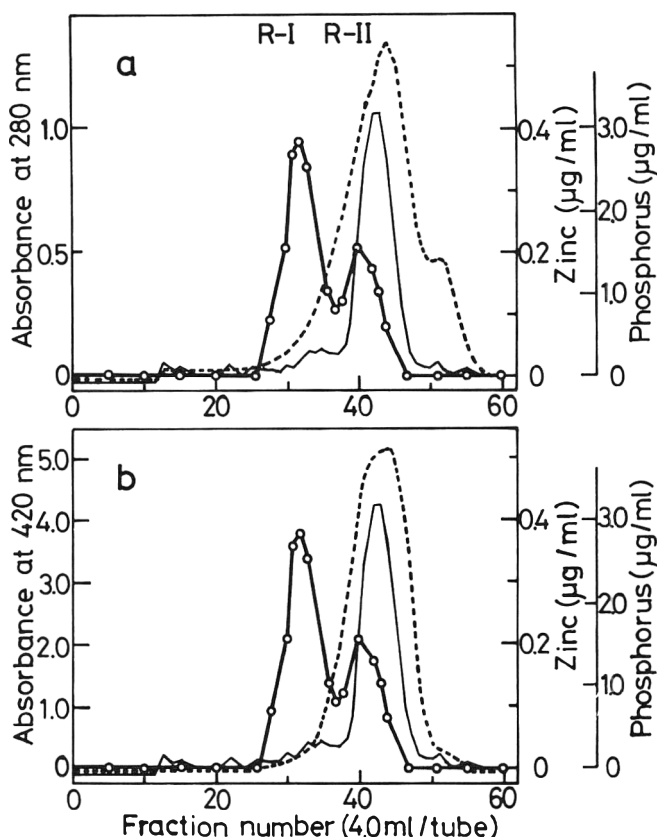
Fig. 2—Gel filtration patterns of the soluble zinc-fraction occurring from the in vitro digestion of polished rice. The zinc peak fractions obtained by the chromatographic procedure were designated as the fractions R-I and R-II according to their order of elution. ——○—— zinc; ———, phosphorus; ·······, absorbance at 280 nm (in a); and ·······, absorbance at 420 nm after incubation with TNBS (in b).

that soluble zinc, as it combines with protein-like substances including phosphorus, may occur in the digesta of beef. This zinc-fraction was designated as fraction B.

The soluble zinc in the digesta of polished zinc comprised 33.6 ± 4.1% (means ± S.D.; n=4) of the total zinc content (Fig. 2). Two zinc fractions, designated as fractions R-I and R-II according to their order of elution, were obtained (Fig. 2). In comparison with the fraction R-II, R-I contained comparatively less protein and phosphorus. R-I also had a detectable amount of carbohydrate as determined by the phenol-sulfuric acid procedure, whereas R-II did not contain detectable amounts of carbohydrate. These zinc-fractions showed typical protein UV absorption patterns. Polyacrylamide gel electrophoresis of the zinc-fractions showed that the majority of zinc was found in protein bands (data not shown).

Molecular weight and amino acid compositions

The molecular weights of the three zinc fractions, B, R-I and R-II, were estimated by gel filtration on Sephadex G-50 to be 1,270, 5,130 and 1,650 dalton, respectively (Fig. 3).

The amino acid profiles of the three zinc-fractions, B, R-I and R-II, were relatively similar to each other (Table 1). L-Aspartic acid, L-glutamic acid and glycine residues were high in all the fractions. In addition, a low level of L-tyrosine, L-phenylalanine and L-histidine residues characterized the three zinc-fractions.

Zinc-binding capacity

The effects of various metal-complexing agents on the relative affinity of the fraction R-I for zinc are shown in Table 2. L-Histidine, L-cysteine and EDTA were shown to prevent the fraction R-I from binding zinc. It is noticeable that picolinic acid and citrate had a weak effect on the zinc binding of R-I.

The effects of these metal-complexing agents on the fractions B and R-II were also determined. Dialysis of the control solutions without the metal-complexing agents resulted in the release of around 70-80% of zinc from the inner solution to the outer. No further experiments were conducted to determine whether or not this phenomenon was ascribable to the dialyzable properties of these two fractions themselves.
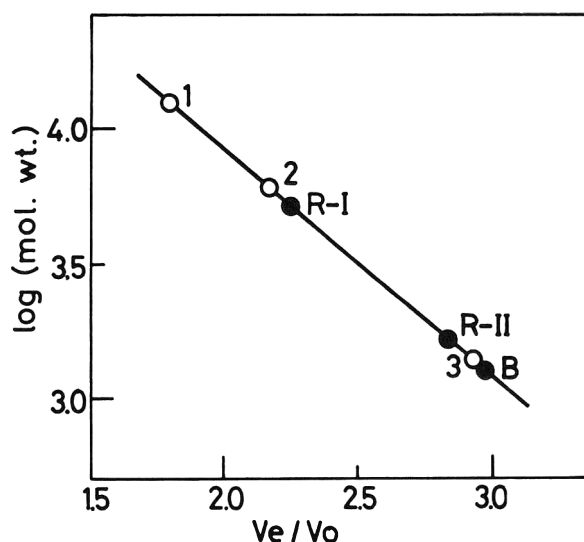
Fig. 3—Determination of the molecular weight of the zinc-bound fractions (B, R-I and R-II) obtained from the digestion of both beef meat and polished rice by molecular-sieving using Spehadex G-50. Standard proteins and their molecular weight are: 1, cytochrome c (12,384); 2, insulin (6,000); and 3, bacitracin (1,411).

The binding capacity of the three zinc fractions toward zinc was estimated by gel filtration chromatography on Sephadex G-10 (Fig. 4). Zinc hydroxide was substantially not found under the conditions employed. The three elution patterns from the fractions B, R-I and R-II were remarkably close to each other (Fig. 4). The zinc concentration at the plateau level was 0.43 ± 0.02 μg/mL. From these elution profiles, the maximum zinc-binding capacity was estimated to be 11.8 mg zinc per 1 mg of protein for the fraction B; 32.9 mg for the fraction R-I; and 5.1 mg for the fraction R-II.

DISCUSSION

THE PRESENT STUDY indicates that most of the soluble zinc formed by the in vitro digestion of beef and polished rice combines with proteinaceous substances (Fig. 1, 2 and 3). In a previous paper, I have also demonstrated that most of the soluble zinc formed by the peptic and tryptic digestion of beef, as analyzed by the combined procedure of chromatography and electrophoresis, was bound to protein-like components with molecular weight of 10,000 dalton or over (Ikeda, 1980). The affinity of the zinc-containing protein-like components for zinc was shown to be considerably high (Table 2 and Fig. 4). These findings suggest that dietary zinc, as it combines with proteinaceous substances that originate in the intermediate metabolism of dietary proteins, may be transported into the intestinal lumen before absorption. Proteinaceous substances occurring upon the digestion of foods presumably exert a profound influence upon zinc absorption. Although there are several reports on the form and nature of the zinc present in raw foods (Harzer and Kauer, 1982; Hazell, 1982), the chemical form of dietary zinc in the alimentary tract is unknown.

Table 1—Amino acid compositions of Zn-binding fractions

Amino acid[a]	Beef (B) mole %	Rice (R-I) mole %	Rice (R-II) mole %
Aspartic acid	9.94	11.72	8.53
Threonine	5.80	5.61	3.85
Serine	6.27	6.43	7.09
Glutamic acid	13.19	14.37	11.35
Proline	3.31	5.76	3.16
Glycine	10.96	13.31	7.81
Alanine	9.46	7.59	8.67
Valine	7.87	7.99	10.44
Methionine	2.37	2.33	2.51
Isoleucine	4.89	4.19	4.25
Leucine	7.59	5.79	7.81
Tyrosine	0.62	0.92	1.56
Phenylalanine	1.51	2.33	3.55
Lysine	10.64	6.50	6.95
Histidine	2.28	1.91	2.85
Arginine	3.39	3.24	9.62

[a] Cysteine and tryptophan were not determined.

Table 2—Influence of metal-complexing agents on the zinc to rice zinc-fraction (R-I) binding

Complexing agent in the buffer	% Zn released from peptides	
	1 mM	0.1 mM
Histidine	85.9	46.0
Cysteine	83.1	77.4
EDTA	87.1	71.8
Picolinic acid	69.4	54.4
Citrate	45.7	18.7
Tris-HCl buffer as control	18.7	
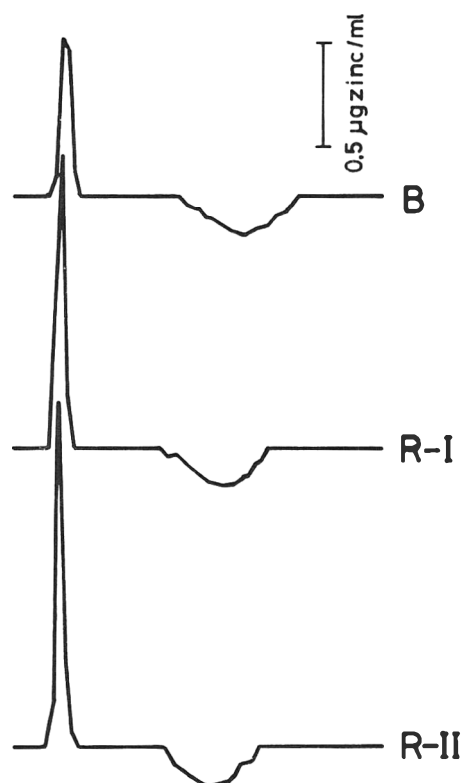
Fig. 4—Elution profiles of zinc obtained by the chromatography of the zinc-free B, R-I and R-II fractions on Sephadex G-10. Elution rate, 45 mL/hr; fraction volume, 2 mL/tube.

Evidence for the occurrence of zinc binding ligands, which may facilitate the intestinal absorption of dietary zinc, have accumulated in recent years. However, their definite relationship to the zinc absorption is the subject of much controversy. Studies with human milk and animals have identified several zinc binding ligands, i.e., picolinic acid (Evans and Johnson, 1980), citrate (Lönnerdal et al., 1980), prostaglandin E_2 (Song and Adham, 1978) and metallothionein (Richards and Cousins, 1975).

On the other hand, several amino acids, including L-histidine and L-cysteine, have been shown to have high affinity for zinc ion (West and Greger, 1978). This suggests that dietary zinc can be easily bound to peptides or amino acids in the alimentary tract. High binding ability of L-histidine and L-cysteine for zinc was also confirmed by the present investigation (Table 2). In addition picolinic acid and citrate, which are considered to be the so-called zinc binding ligands, were found to exhibit rather low affinity for zinc. On the other hand, it is well known that chelating activities of many of metal-complexing agents generally are highly dependent upon pH. Therefore, changes in pH, which parallel physiological conditions in the alimentary tract, appear to considerably affect mineral binding capacities of the above metal-complexing agents.

Amino acid analyses revealed that the soluble zinc-fractions occurring in the in vitro digestion of the two foods examined were high in the acidic amino acids and glycine, and lower in L-histidine (Table 1). This fact suggests that the presence of the acidic amino acid residues in these proteinaceous substances, but not the metal-complexing amino acid residues, appears to be essential for the zinc attraction. On the other hand, there are a variety of dietary substances, which may have high affinity for zinc, e.g., amino acids, organic acids and indigestible fiber, in the in-

testinal fluids or contents. The question whether or not dietary zinc is bound only toward a specific ligand before absorption remains unanswered. If zinc uptake across the luminal brush border into the mucosal cell is enhanced by the so-called zinc-binding ligands, an important consideration will ultimately be the source of the ligands. It is uncertain whether these ligands occur directly from dietary constituents, or whether they are synthesized in the intestinal cells and other tissues. Further characterization of the zinc-bound substances occurring upon the digestion of foods will allow an exact evaluation of the bioavailability of dietary zinc. Research is also needed to clarify the chemical form of dietary zinc which is most absorbable after digestion.

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Study of the Influence of Solute (n-Alcohols and n-Alkanes) Chain Length on Their Retention by Purified Olive Oil

A. LEBERT and D. RICHON

——————ABSTRACT——————

An apparatus, which was especially designed to measure the activity coefficients of infinitely dilute solutes, has been used to determine the effect of increasing the solute chain length on the ability of solvents to retain solutes. In this way, measurements of activity coefficients have been performed 298.1, 308.1 and 328.1°K for pentane to decane and methanol to hexanol infinitely diluted in purified olive oil.

INTRODUCTION

ALTHOUGH the main function of food is nutritive, its cost and sensory qualities also have an important influence on consumer choice. As aroma is an essential sensory quality, it is often necessary either to optimize its retention during food processing or to aromatize neutral foods (from an olfactive point of view). Such an optimization depends closely on physicochemical mechanisms and especially on thermodynamic properties because molecular interactions play an important role along with diffusion phenomena. The thermodynamic quantity which is the most relevant to that study is the infinite dilution activity coefficient.

Several different methods have been used up to date to determine infinite dilution activity coefficients: head space analysis methods (Buttery et al., 1971; Scatchard, 1964; Voilley et al., 1977; Kolb, 1980) and mutual solubility measurement methods (Le Maguer, 1981). Compared to the stripping method developed recently by Leroi et al. (1977), these methods tend to be inaccurate and time consuming, and, in some case, unsuitable. In this work, the stripping method has been used with the equilibrium cell designed by Richon and Renon (1980); measurements have been carried out on alkanes and alcohols with olive oil. Alkanes and alcohols have been chosen as models because of their high polarity differences. Lipids are common substances in food science; they are precursors of numerous aromas. They can interact with taste ingredients such as salts and with bitter or sour agents contained in saliva. They are extensively used as nonpolar solvents in the food industry. In this work, olive oil was selected as an example of lipid substances. In the near future, other solutes and other solvents will be involved in our studies. All these new results will extend the necessary data base to develop new thermodynamic models and then predict thermodynamic properties for a large amount of mixtures constituting food.

MATERIALS & METHODS

Methods

A stirred solute-solvent mixture is kept at constant temperature in an equilibrium cell immersed in a liquid bath. The mass fraction

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of the solute in the mixture is always much less than 10^{-4}. About 10 µL solute are introduced directly, using a syringe, into the solvent. The mass of solvent (approximately 30g) is determined within 2.10^{-4} g by weighing the equilibrium cell empty and with the solvent. A constant carrier gas flow is introduced into the equilibrium system through a ten capillary tube injector leading to formation of small carrier gas bubbles, which strip the components of the liquid mixture into the vapor phase. The vapor phase leaving the cell is periodically sampled using a thermostated 6-way gas sampling valve (Fig. 1) and analyzed with a gas-liquid chromatograph (Girdel, model 3000) equipped with a differential flame ionization detector. The equilibrium cell was described in Richon and Renon (1980). Its design resulted from a mass transfer study developed by Richon et al. (1980). It has been shown that the solute chromatographic peak area variation (for a nonvolatile solvent) is an exponential function of time provided that the detector response is linear (see Appendix 1). Then, an infinite dilution activity coefficient can be derived from determination of this function and some measurements: total pressure at equilibrium, carrier gas flow and total amount of solvent in the still. No calibration of the chromatograph is necessary.

Then, the expression which relates the activity coefficient to the measured quantities is:

$$\gamma_s^\infty = \left(+\frac{1}{t} \ln \frac{(S_s)_{t=0}}{(S_s)_t} \right) \frac{n_S RT}{DP_s^S}$$

Materials

Commercial virgin olive oil was purified by adding 15 volume percent olive oil to 85 volume percent chloroform then separating on alumina powder.

The chloroform was evaporated under a vacuum. This eliminated free acids, mono and diglycerides, phospholipids and pigments. The olive oil had the following composition: palmitic acid — 22.1%;
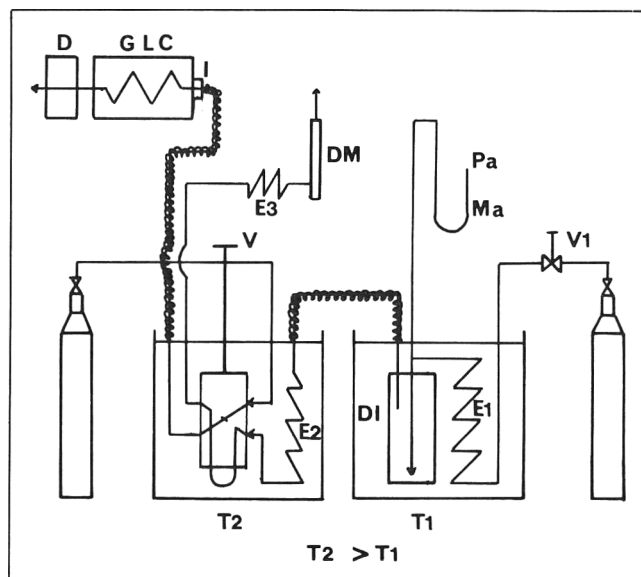
Fig. 1—Flow diagram of the dilutor apparatus: D = detector of the chromatograph; DI = Dilutor; DM = Bubble flowmeter; $E_1E_2E_3$ = Thermal exchangers; GLC = Gas liquid chromatograph; I = Injector; Ma = Hg manometer; Pa = Atmospheric pressure; ∿∿∿ = Heating resistance; T = Temperature; V_1 = 3-way valve; V_2 = Needle valve; V_3 = 6-way gas sampling valve.

palmitoleic acid — 1%; stearic acid — 5.3%; oleic acid — 62.1%; linoleic acid — 9.5%. There were also some ppm linolenic, arachidic and arachidnic acids present.

The origin and purity of the solutes, alkanes and alcohols, are given in Table 1.

RESULTS & DISCUSSION

EXPERIMENTAL RESULTS (activity coefficient data) appear in Tables 2 and 4 along with excess partial molar enthalpy deduced from activity coefficient data (see Appendix 2).

Alcohol-purified olive oil systems

Deviations from the ideal case are positive and an increasing function of the alcohol chain length (Table 2). Error in each activity coefficient value was estimated from the dispersion of at least five determinations. Then, as the infinite dilution activity coefficient is greater than 1, the concentration of alcohols in the vapor phase at equilibrium was higher than it would be in the case of an ideal solution.

At any temperature, the infinite dilution activity coefficient data satisfy the following set of inequalities:

$$\ln\gamma^{\infty}_{methanol} > \ln\gamma^{\infty}_{ethanol} > \ln\gamma^{\infty}_{propanol} > \ln\gamma^{\infty}_{butanol}$$
$$> \ln\gamma^{\infty}_{pentanol} > \ln\gamma^{\infty}_{hexanol}$$

Under these conditions, hexanol was the alcohol in the series studied that gave the solution closest to an ideal solution when used with olive oil. This behavior is a consequence of the diminishing effect of the polar group with respect to the increasing alkyl chain length.

Infinite dilution activity coefficients are compared with those from Allaneau (1979) (Table 3). The latter obtained finite dilution activity coefficient data through an head space analysis method and extrapolated them at infinite dilution using several two-parameter models, namely,

UNIQUAC (Abrams and Prausnitz, 1975), N.R.T.L., equations of Wilson and Van Laar (for these last three models, see Prausnitz, 1969). The data from Allaneau (1979) are in general lower than those reported here by about 20%. Three explanations can be proposed for this discrepancy: (1) the olive oil used by Allaneau was not purified and contained polar components such as mono and diglycerids, which could interact strongly with polar components such as alcohols; (2) the head space analysis methods could lead to experimental errors because of the difficulty of reaching equilibrium conditions; (3) the models were not good enough to give accurate extrapolations at infinite dilution.

Plots of $\ln\gamma^{\infty}_{s}$ vs $1/T$ (Fig. 2) gave straight lines; thus, in the small temperature range studied here, the excess partial molar enthalpy appeared as temperature independent and could be calculated easily from the slope of the curves. For all the series of alcohols considered, the excess partial molar enthalpies could be represented by the mean value: 5900 $J \cdot mol^{-1}$ (Table 2).

Alkane-purified olive oil systems

For these systems, deviations from the ideal case were negative, in contrast to the behavior of alcohol-purified olive oil systems (Table 4). Errors in activity coefficient values were estimated from the dispersion of at least five experimental determinations. We note the solutions approached the ideal more closely as the alkane chain length increased (when the alkane chain length was increased the size difference between the alkane and olive oil decreased).

Values at 298.1°K from Allaneau (1979) (Table 5) are different from those reported here by about 10%. These differences cannot be explained by the fact that Allaneau used a nonpurified olive oil leading to polar-polar interaction, because alkanes are nonpolar components. The only possible explanations are related to the inaccuracy of the extrapolation models or to experimental errors. The dilutor technique allows activity coefficient measurements within 1–3% (Leroi et al., 1977; Richon et al., 1980), depending on the volatility of the solutes (the most accurate measurements were obtained with the smallest solutes of each homologous series).

Table 1—Origin and purity of the solutes

Solutes	Origin	Minimum certified purity in percent
Methanol	Carlo Erba	99.9[a]
Ethanol	Carlo Erba	99.9[a]
n-Propanol	Carlo Erba	99.9[a]
n-Butanol	Prolabo	99.5[b]
n-Pentanol	Prolabo	99.0[b]
n-Hexanol	Prolabo	98.0[b]
n-Pentane	Merck	99.7[b]
n-Hexane	Merck	99.7[b]
n-Heptane	Merck	99.7[b]
n-Octane	Prolabo	99.8[b]
n-Nonane	Prolabo	99.6[b]
n-Decane	Prolabo	99.4[b]

[a] The purity is expressed in volume percent of the solute contained in the commercial product.
[b] The purity is expressed in weight percent of the solute contained in the commercial product.

Table 3—Comparison at 298.1°K of experimental results to those from literature

Solutes	$\gamma^{\infty a}$	$\gamma^{\infty b}$			
		Van Laar	Wilson	N.R.T.L.	UNIQUAC
Methanol	3.21	2.59	2.66	2.56	2.59
Ethanol	2.53	2.11	2.16	2.06	2.08
Propanol	2.36	1.91	1.94	1.89	1.85
Butanol	2.26	1.93	1.96	1.61	1.86

[a] This work
[b] from Allaneau (1979). Extrapolated results from experimental finite dilution activity coefficients using different models.

Table 2—Infinite dilution excess partial molar enthalpy and activity coefficients at different temperatures for alcohols dissolved in purified olive oil

Solutes	$\gamma^{\infty a}$				$\bar{h}^{E\infty b}$
	T=298.1°K	T=308.1°K	T=318.1°K	T=328.1°K	(kJ·mol^{-1})
Methanol	3.21 ± 0.04	2.96 ± 0.04	2.76 ± 0.03	2.60 ± 0.03	5.7 ± 0.2
Ethanol	2.53 ± 0.04	2.35 ± 0.05	2.17 ± 0.04	2.04 ± 0.04	5.9 ± 0.2
Propanol	2.36 ± 0.04	2.17 ± 0.04	2.02 ± 0.04	1.91 ± 0.03	5.7 ± 0.2
Butanol	2.26 ± 0.04	2.10 ± 0.05	1.97 ± 0.05	1.82 ± 0.05	5.8 ± 0.3
Pentanol	2.21 ± 0.05	2.06 ± 0.06	1.91 ± 0.06	1.77 ± 0.05	6.0 ± 0.4
Hexanol	2.17 ± 0.07	2.03 ± 0.07	1.87 ± 0.06	1.74 ± 0.07	6.0 ± 0.5

[a] γ^{∞} is the infinite dilution activity coefficient.
[b] $\bar{h}^{E\infty}$ is the excess partial molar enthalpy at infinite dilution.

Table 4—Infinite dilution excess partial molar enthalpy and activity coefficients at different temperatures for alkanes dissolved in purified olive oil

Solutes	$\gamma^{\infty a}$				$\bar{h}^{E\infty b}$
	$T = 298.1°K$	$T = 308.1°K$	$T = 318.1°K$	$T = 328.1°K$	$(kJ\cdot mol^{-1})$
n-Pentane	0.632 ± 0.007	0.637 ± 0.011	0.642 ± 0.010	0.646 ± 0.011	−0.60 ± 0.03
n=Hexane	0.709 ± 0.007	0.715 ± 0.012	0.720 ± 0.013	0.725 ± 0.012	−0.60 ± 0.04
n-Heptane	0.759 ± 0.012	0.765 ± 0.013	0.770 ± 0.016	0.776 ± 0.014	−0.59 ± 0.07
n-Octane	0.797 ± 0.016	0.803 ± 0.015	0.809 ± 0.011	0.815 ± 0.017	−0.61 ± 0.10
n-Nonane	0.824 ± 0.019	0.829 ± 0.021	0.836 ± 0.022	0.852 ± 0.019	−0.59 ± 0.14
n-Decane	0.846 ± 0.027	0.849 ± 0.024	0.857 ± 0.028	0.863 ± 0.023	−0.58 ± 0.16

[a] γ^{∞} is the infinite dilution activity coefficient.
[b] $\bar{h}^{E\infty}$ is the excess partial molar enthalpy at infinite dilution.

Fig. 2—Logarithm of the infinite dilution activity coefficient as a function of the inverse of temperature for different solutes in purified olive oil: + Decane; x Nonane; ▲ Octane; ● Heptane; ▼ Hexane; • Pentane.

Excess partial molar enthalpies for n-alkanes in olive oil were calculated from the slopes of $\ln\gamma^{\infty}$ vs $1/T$ (Fig. 3), and the results appear in Table 4. For the six n-alkanes in olive oil, excess partial molar enthalpies were approximately the same with a mean value of about -600 $J\cdot mol^{-1}$.

CONCLUSION

IT HAS BEEN SHOWN that the dilutor technique is very well suited to measure infinite dilution activity coefficients of light solutes in olive oil. Determinations were accurate to within 3% in the worst cases (big solute molecules) and within 1% for the small solute molecules, which have the highest vapor pressures.

Consequently, as well as improving our knowledge about solute retention, this method allows us to calculate the heats of mixing. In the infinite dilution range the mixing of n-alcohol and purified olive oil was endothermic while the n-alkane-olive oil mixing was exothermic. Secondly, knowing the olfactive lower levels of detection of volatile components and their activity coefficients in one lipid substance, it is possible to calculate the olfactive lower levels of detection of these volatile components in other lipid substances by only determining their activity in these other lipids (Lebert, 1980). Infinite dilution activity coefficient data are then relevant thermodynamic quantities for optimizing the processes of aromatization or trying to find the best ways of increasing aroma retention during food processing. An extensive use of the new method to obtain accurate activity coefficient data quickly would be particularly useful to the food industry.

Table 5—Comparison at 298.1°K of experimental results to those from literature

Solutes	$\gamma^{\infty a}$	$\gamma^{\infty b}$			
		Van Laar	Wilson	N.R.T.L.	UNIQUAC
Pentane	0.63	0.50	0.49	0.46	0.49
Hexane	0.71	0.59	0.58	0.57	0.58
Heptane	0.76	0.60	0.59	0.56	0.59
Octane	0.80	0.64	0.60	0.58	0.62

[a] This work
[b] From Allaneau (1979). Extrapolated results from experimental finite dilution activity coefficients using different models.

NOTATION

D   carrier gas flow (pressure P, temperature T) (cm^3/min)
D' total gas flow at dilutor exit (pressure P, temperature T) (cm^3/min)
h   molar enthalpy $(J\cdot mol^{-1})$
H   Henry's constant (atm)
n   number of moles
P   total pressure (atm)
R   gas constant $(cm^3\cdot atm\cdot g\cdot mol^{-1})$
S   chromatographic peak area
t   time (min)
T   temperature $(°K)$
v   volume (cm^3)
x   liquid mole fraction
y   vapor mole fraction

Greek letters

γ activity coefficient
ϕ fugacity coefficient

Subscripts

i   component i
s   solute
S   solvent
CG carrier gas

Superscripts

E   excess property
LS saturated liquid
S   saturated property
∞   infinite dilution property
−   partial property

APPENDIX 1

THEORY OF THE DILUTOR TECHNIQUE

If the vapor-liquid mixture in the cell is in equilibrium, the equilibrium equations for each component are:
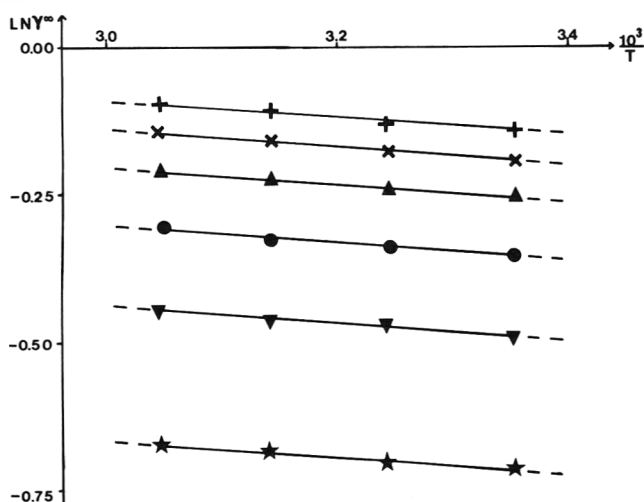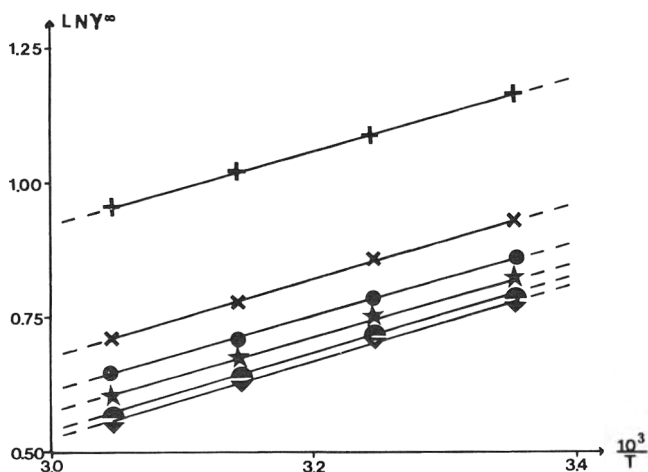
Fig. 3—Logarithm of the infinite dilution activity coefficient as a function of the inverse of temperature for different solutes in purified olive oil: + Methanol; x Ethanol; ● n-Propanol; ✶ n-Butanol; ▲ n-Pentanol; ▼ n-Hexanol.

$$x_s \gamma_s \, P_s^S \phi_s^S \exp\left(\frac{v_s^{LS}(P-P_s^S)}{RT}\right) = y_s \phi_s P$$

$$x_S \gamma_S \, P_S^S \phi_S^S \exp\left(\frac{v_S^{LS}(P-P_S^S)}{RT}\right) = y_S \phi_S P \qquad (1)$$

$$x_{CG} H_{CG} = y_{CG} \phi_{CG} P$$

where solutes = s; solvent = S; and carrier gas = CG.

When the solute is highly diluted in the solvent, and if the solubility of the carrier gas in the liquid mixture is neglible, γ_s^∞ is equal to γ_s and γ_S is very close to 1. Working at atmospheric pressure, vapor phase corrections can be neglected. Then, the set of Eq. (1) reduces to:

$$x_s \gamma_s P_s^S = y_s P$$

and

$$P_S^S = y_S P \qquad (2)$$

n_s and n_S are the total numbers of moles of solute and solvent in the cell at time t. The quantities withdrawn from the liquid mixture $(-dn_s)$ and $(-dn_S)$ by the carrier gas are:

$$dn_s = -y_s P \frac{D'dt}{RT} \qquad (3)$$

and

$$dn_S = -y_S P \frac{D'dt}{RT} \qquad (4)$$

D' is the total volumetric flow rate of gas flowing outside the cell at pressure P and temperature T.

An overall mass balance around the equilibrium cell gives:

$$D' = D - \frac{RT}{P}\left(\frac{dn_s}{dt} + \frac{dn_S}{dt}\right) \qquad (5)$$

Combining Eq. (2), (3), (4), and (5) and assuming $x_s = n_s/n_S$ (infinite dilution) yields:

$$\frac{dn_s}{dt} = -\frac{n_s}{n_S} \gamma_s^\infty P_s^S \cdot A \qquad (6)$$

and

$$\frac{dn_S}{dt} = -P_S^S \cdot A \qquad (7)$$

with A = $\dfrac{1}{1 - \dfrac{n_s}{n_S} \gamma_s^\infty \dfrac{P_s^S}{P} - \dfrac{P_S^S}{P}}$

In general, A is very close to 1. Under these conditions, integration of Eq. (6) for the case of a nonvolatile solvent gives:

$$\ln \frac{n_s}{(n_s)_{t=0}} = -\frac{D}{RT} \frac{P_s^S}{n_S} \bar{\gamma}_s^\infty t \qquad (8)$$

If the chromatographic detector response is linear, n_s is a linear function of solute chromatographic peak area, S_s, and then we get:

$$\gamma_s^\infty = \left(+\frac{1}{t}\ln\frac{(S_s)_{t=0}}{(S_s)t}\right) \cdot \frac{n_S RT}{DP_s^S} \qquad (9)$$

APPENDIX 2

The temperature dependence of the activity coefficient is given by:

$$\left(\frac{\partial \ln\gamma_i}{\partial T}\right)_{P,n_{j\neq i}} = -\frac{\bar{h}_i^E(T)}{RT^2} \qquad (10)$$

and $\bar{h}_i^E$ is the excess partial molar enthalpy.

At infinite dilution, relation A10 becomes:

$$\left(\frac{\partial \ln\gamma_i}{\partial T}\right)_{P,n_{j\neq i}} = -\frac{\bar{h}_i^{E,\infty}(T)}{RT^2} \qquad (11)$$

If we suppose that in the small temperature range studied in this work, the enthalpy temperature dependence is negligible, then, integration of Eq. (11) leads to:

$$\ln\gamma_i^\infty = \frac{\bar{h}_i^{E,\infty}}{RT} + c^{te} \qquad (12)$$

From Eq. (12), it is clear that if the above assumption is satisfied, plotting $\ln\gamma_i^\infty$ vs $1/T$ will result in straight lines from which values of $\bar{h}_i^{E,\infty}$ can be easily deduced.

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Enzymatic Modification of Fish Frame Protein Isolate

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────────────── ABSTRACT ──────────────

Protein isolate was hydrolyzed with pepsin yielding an approximate degree of hydrolysis of 80%. Frame protein hydrolysates were then employed for plastein synthesis. Enzyme induced plastein was optimized at: (1) pH 5 for pepsin and pH 7 for alpha-chymotrypsin; (2) substrate concentration, 40% w/v; (3) time of incubation, 24 hr; (4) enzyme/substrate ratio, 1:100 w/w. Percent plastein yields of 33.8 and 27.3 were found for pepsin and alpha-chymotrypsin, respectively, when trichloracetic acid was used as the precipitating agent. However, when plastein was precipitated by ethanol, the percent yield was found to be 46 and 40.5 for pepsin and alpha-chymotrypsin, respectively.

INTRODUCTION

NEW PROTEIN SOURCES from oilseed and vegetable proteins, single cell proteins, leaf proteins and fish proteins are relatively abundant, easy to prepare and relatively inexpensive yet are unused because of their poor functional performance in foods. Clearly, the widespread use of novel proteins involves the development of the technologies for the efficient extraction and preparation of these proteins, i.e. fractionation and refinement. Of paramount importance, however, is the development of the technologies to modify the physicochemical properties of these proteins so that they may successfully perform specific functions in foods and yet be nutritious and nontoxic. One approach of altering the functional properties of protein is by enzymatic modification. This method involves limited hydrolysis of the protein with selected proteolytic enzymes to split specific peptide bonds. Along with a decrease in size of the protein, there are changes in solubility and functional characteristics.

A further novel approach to alter the physicochemical, functional and nutritional properties is application of the plastein reaction. A protein is enzymatically hydrolyzed an and the subsequent hydrolysate is incubated with certain proteolytic enzymes under high substrate conditions. Hydrolysis is therefore reversed and gelled protein-like products, plasteins, are formed whose properties are different from the original proteins. Application of the plastein reaction to enzymatic hydrolystates of novel proteins may give rise to products with interesting or enhanced functional and/or nutritional properties which may be of great benefit in the formulation of new food products.

A protein isolate prepared by Montecalvo et al. (1984) from flounder frames after filleting was used as the substrate for plastein formation in this study. Those factors affecting the rate and extent of plastein formation, such as the specificity of the enzyme, the extent of denaturation of the protein substrate as well as substrate and enzyme concentration and pH of reaction environment were studied.

MATERIAL & METHODS

Hydrolysis of protein isolates

The method of Yamashita et al. (1970) and Edwards and Shipe (1978) was used for the preparation of hydrolysates used as substrates for the plastein reaction. In all cases, batch hydrolysis was employed by dispersing 30g flounder frame protein isolate (81.5% protein, 10.6% lipid, 0.9% ash and 3.7% moisture; prepared according to Montecalvo et al., 1984 into 2.89L distilled water. The pH of the solution was adjusted to 1.65 with the addition of 110 mL 2M HCl. Then 300 mg pepsin (2x crystallized, Sigma) was carefully solubilized in 10 mL distilled water and slowly added to the substrate solution to give an effective enzyme: substrate ratio of 1:100 on a w/w basis. The hydrolysate solution was incubated at 37°C with slow agitation. After 24 hr, the pH was adjusted to pH 7 with 2M NaOH and stirred at room temperature for an additional 2 hr to inactivate residual pepsin activity. The hydrolysate was then centrifuged at 5000 rpm (RCF = 3016 g) for 10 min and followed by filtering through Whatman #1 filter paper to remove free lipids. The filtrates were then freeze-dried, collected into air-tight bottles and stored at 5°C.

Determination of the degree of hydrolysis

This method is based on the ratio percent of trichloracetic acid (TCA) soluble nitrogen, as discussed by Yamashita et al. (19870) and Edwards and Shipe (1978). During the course of hydrolysis, 50 mL aliquots were removed after 1, 4, 8, 12, 18, 24 and 48 hr. At each time period, triplicate samples wer taken and 50 mL 20% TCA was added to create a 10% TCA soluble and TCA insoluble fractions. The solutions were centrifuged at 10000 rpm (RCF = 12064g) for 15 min and the supernatants were assayed for nitrogen by the Kjeldahl method. Samples taken directly from the hydrolysate were diluted and assayed for nitrogen. The approximate degree of hydrolysis is expressed as:

$$\text{Approx degree of hydrolysis} = \frac{10\% \text{ TCA soluble N}}{\text{Total N}} \times 100$$

Determination of the approximate average peptide size

The method of Kang and Rice (1970) was used. During the course of peptic hydrolysis at 1, 4, 8, 12, 24 and 48 hr, 20 mL aliquots of hydrolysate were removed and centrifuged at 5000 rpm (RCF = 3016g) for 15 min. The supernatants were filtered through Whatman #1 filter paper into 100 mL volumetric flasks and the volumes brought up to 100 mL with the addition of distilled water. All Flasks were mixed well and a mL aliquots were diluted with water and assayed for total soluble nitrogen. Another 1 mL aliquot was diluted and assayed for alpha amino nitrogen. The following equation was used to compute the average peptide chain length:

$$\frac{\mu g \ \alpha \text{ amino N}}{\mu g \text{ soluble N}} = \frac{1}{\text{avg peptide chain length}}$$

Plastein assay optimization

Plastein has been defined by Fugimaki et al. (1977) as the increase in 10% TCA insoluble material during incubation of a low molecular weight hydrolysate at a concentration greater than 20% w/v with a synthesis enzyme such as pepsin or alpha-chymotrypsin.

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Methods of plastein assay are based on measuring the increase in 10% TCA or 50% ethanol insoluble precipitations formed during plastein systhesis. The method used for plastein assay was developed by Fugimaki et al. (1977), Yamashita et al. (1970) and later modified by Hofsten and Lalasidis (1976).

Plastein synthesis. 4g freeze-dried frame protein hydrolysate (incubated 24 hr with pepsin) was dissolved in 9.7 mL distilled water creating a 40% (w/v) substrate concentration. The pH was adjusted to 5 with the addition of 0.3 mL 2N HCl as previously determined by trial runs. Then, 40 mg pepsin were added and mixed into the substrate solution. Controls containing no enzyme or no substrate were also prepared. All beakers were incubated at 37°C without any agitation for 24 hr, after which a solid gel was formed. Then 10 mL 20% TCA was added to each beaker and mixed with the aid of a small glass rod to disrupt the gel and to precipitate plastein. Both TCA treated plastein samples and controls were centrifuged at 5000 rpm (RCF = 3016g) for 15 min to separate the TCA insoluble fraction from the TCA soluble fraction (supernatant). TCA insoluble material was removed from the centrifuge tubes with the aid of small amounts of distilled water and freeze dried. The white freeze dried TCA insoluble material was collected into small plastic vials and used as the source of plastein for the standardization of the turbidometric assay.

Turbidometric plastein assay. A stock solution of plastein was prepared by suspending 10 mg freeze-dried plastein in 5 mL 10% TCA. The suspension was homogenized in a Potter-type hand homogenizer with 5 up and down strokes to create an even suspension. The stock plastein solution was then serially diluted with 10% TCA to yield 0.05, 0.1, 0.2, 0.4, 0.8 and 1 mg/mL concentrations. Each standard was well mixed and the absorbance of each standard was measured on a Gilford Model 240 Spectrophotometer at 660 nm. A reagent blank containing 10% TCA was used to adjust the slit width. A plot of absorbance vs concentration was made. Absorbance values for plastein samples were calculated as follows: Plastein productivity = Plastein abs. at 660 nm − Abs. at 660 nm of substrate and/or enzyme control.

Effect of time on stabiliy of TCA-plastein. Three concentrations of plastein standards containing 0.8, 0.6 and 0.05 mg/mL, representing a high, intermediate and low concentration, were assayed after 0, 5, 10, 15, 30 and 60 min of standing at room temperature to assess any changes in absorbance values during the assay of large numbers of samples.

Effect of pH on plastein synthesis. The optimum pH for both pepsin induced plastein and alpha-chymotrypsin induced plastein was determined by adjusting the pH of 40% w/v substrate solutions (prepared by solubilizing 1.0g peptic hydrolysate in 2.5 ml water) to 2, 3, 4, 4.5, 5, 6, 7, 8, 9 and 10 with small amount sof 0.5N HCl or 0.5N NaOH. The amount of acid or alkali used to adjust the substrate solutions was determined from trial runs so as to not affect the substrate concentration. A total of 10 mg of each enzyme was added to a series of pH adjusted substrates creating a 1:100 enzyme-substrate ratio based on weight of enzyme to that of substrate. A 0.5 ml aliquot from each incubation mix was carefully pipetted into 3 mL assay tubes, in triplicate, capped with parafilm and incubated at 37°C for 24 hr, Additionally, 0.1 mL of toluene was added to each tube to control microbial growth during the incubation period. Immediately following incubation, 0.5 mL 20% TCA was added to each tube to precipitate plastein. Also, 0.5 mL 20% TCA was added to both substrate and enzyme control. All tubes were then centrifuged at 5000 rpm (RCF = 3016g) for 10 min. The TCA precipitated pellets were then homogenized with 1 mL 10% TCA. Then, 0.05 mL aliquots from each tube were mixed with 5 mL 10% TCA and the resulting turbidity read at 660 nm.

Effect of substrate concentration on plastein synthesis. Substrate solutions were prepared by dissolving 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6g of hydrolysate in 1 mL water adjusted to pH 5 for pepsin and pH 7 for alpha-chymotrypsin. Enough of the appropriate enzyme was added to each tube to create a 1:100 enzyme/substrate ratio. All tubes were incubated at 37°C for 24 hr after which each tube was assayed, in triplicate, for plastein using the turbidometric procedure.

Effect of enzyme concentration on plastein synthesis. 40% substrate solutions were prepared by dissolving 1.0g of 24-hr peptic hydrolysate in 2.5 mL distilled water adjusted to pH 5 for pepsin and pH 7 for alpha-chymotrypsin. Quantities of 5, 10 and 20 mg of enzyme were added to individual substrate solutions resulting in 1:200, 1:100 and 1:50 enzyme/substrate ratios for each enzyme. All tubes were gently mixed and incubated at 37°C. Tubes were re-

moved and assayed for plastein productivity after 0, 1, 2, 4 and 24 hrs of incubation. Resulting plastein productivity was measured by the turbidometric method.

Trichloroacetic acid and ethanol yields of plastein. 10g amounts of 24-hr peptic hydrolysate were solubilized in 25 mL of distilled water. The pH of substrate solutions were adjusted to 5 for pepsin and 7 for alpha-chymotrypsin. 100 mg of pepsin was added to duplicate substrate preparations and 100 mg of alpha-chymotrypsin was added to the remaining two substrate preparations. Each enzyme/substrate mix was incubated for 24 hr at 37°C with 1 mL of toluene to prevent microbial activity. 25 mL of cold absolute ethanol was added to one set of pepsin and alpha-chymotrypsin induced plastein samples and 25 mL of 20% TCA were added to the remaining two enzyme/substrate preparations. Precipitates obtained were separated from solution by centrifugation at 5000 rpm (RCF = 3016g) for 15 min. Precipitates were quantitatively removed and transferred to small stainless steel trays with the aid of small amounts of distilled water, frozen at −15°C, freeze-dried, collected and weighed. The percent yield was calculated from the amount of plastein obtained for each enzyme and each precipitating agent. The ratio percent of the amount of protein recovered in terms of plastein vs the original amount of protein in the peptic hydrolysate was calculated by using the following equation:

$$\% \text{ yield} = \frac{\text{amt of protein ppt as plastein}}{\text{amt of protein contained in substrate}} \times 100$$

Proximate analysis of freeze-dried frame protein isolates, hydrolysates and plastein

Protein, moisture, ash, and crude lipid were determined by AOAC procedures (1970).

RESULTS & DISCUSSIONS

Pepsin hydrolysis of flounder frame protein isolates

The degree of hydrolysis of flounder frame protein isolates determined at various incubation times with pepsin is shown in Table 1. Increased hydrolysis was indicated by increased solubility of the hydrolysis in 10% TCA. There was a leveling off of percent hydrolysis after 18 hr of incubation. Upon further incubation, the degree of hydrolysis reached 85.2% after 48 hr. It was assumed that peptides soluble in 10% TCA may be further hydrolyzed at longer incubation times yielding products differing in component molecular weight distributions. Such further hydrolysis would not be reflected by TCA solubility measurements. As hydrolysis of the protein isolate proceeds, those peptides possessing low enough molecular weights to be soluble in 10% TCA may be further hydrolyzed to smaller peptides without affecting the amount of TCA soluble nitrogen. Additional studies would be needed to confirm this assumption.

The freeze-dried yields of hydrolysate after incubation with pepsin at 37°C are shown in Table 2. After 1 hr of hydrolysis, 28.8g of hydrolysate was recovered. At 18 hr of hydrolysis, 35.4g were recovered which contained approximately 60% protein. Further hydrolysis did not substantially increase the yield or the protein content suggesting that hydrolysate yields leveled off after 18 hr of incubation.

Table 1—Approximate degree of hydrolysis of NaOH-HCl flounder frame protein isolate by pepsin

Time of incubation (hr)	Degree of hydrolysis (%)[a]
1	40.6
4	58.6
8	63.7
18	76.6
24	78.5
48	85.2

[a] Degree of hydrolysis = $\dfrac{10\% \text{ TCA soluble N}}{\text{total N}} \times 100$

Table 2—Yields of hydrolysate after incubation at 37°C with pepsin

Time of hydrolysis (hr)	Wt freeze-dried fraction[a]	% N	% Protein
1	28.8	7.56	47.3
4	31.8	9.37	58.6
8	33.1	9.68	60.5
18	35.4	9.71	60.7
24	36.6	9.78	61.1
48	37.2	9.85	61.6

[a] Average of duplicate runs

Table 3—Effect of time of incubation on the average peptide size chain length during pepsin hydrolysis of flounder frame protein isolate

Incubation time (hr)	Soluble nitrogen[a] (μg/ml)	Alpha amino nitrogen (μg/mL)	$\dfrac{\text{Free Amino N}}{\text{Soluble N}} \times 100$	Est. peptide[b] chain length
1	619	45	7.3	13.8
4	775	83	10.7	9.3
8	769	92	12.0	8.4
18	926	143	15.4	6.4
24	948	166	17.5	5.7
48	1030	300	29.1	3.4

[a] Values are the average of triplicate runs
[b] Estimated from the equation: $\dfrac{\text{alpha amino N}}{\text{total sol. N}} = \dfrac{1}{\text{avg peptide chain length (no. of amino acids)}}$

Fig. 1—Effect of pH on plastein production using pepsin and alpha-chymotrypsin. Enzyme/substrate ratio=1:100 w/w; substrate concentration=40% w/v; temperature=37° C.

Fig. 2—Effect of substrate concentration on plastein production using pepsin and alpha-chymotrypsin. Enzyme/substrate ratio= 1:100 w/w; pH=5 for pepsin, pH=7 for alpha-chymotrypsin; temperature=37° C.

The effect of time of incubation on the approximate average peptide chain length during pepsin hydrolysis of frame protein isolate is shown in Table 3. The peptide chain lengths were inversely proportional to the percent free amino nitrogen. While the estimated peptide chain lengths are only approximations, they show the general effects of the extent of hydrolysis as a function of length of incubation and the approximate degree of hydrolysis (Table 1).

Hale (1969) found that after a 24-hr digestion of fish protein concentrate (EPC), a highly denatured substrate, with pepsin at an enzyme/substrate ratio of 1:100, the approximate average peptide chain length was 3.5 amino acid residues. However, after 24 hr of incubation of flounder frame protein isolate under similar conditions, a peptide chain length of 5.7 amino acid residues was obtained (Table 3). The different composition of the protein isolate from flounder frames and protein concentrate from whole fish could affect the rate of enzymatic degradation. The degree of denaturation could also influence the rate and extent of enzymatic protein degradation (Fugimaki et al., 1977).

Optimization of enzymatic plastein production

The optimum pH for plastein synthesis using pepsin was pH 5 (Fig. 1), which is compatible with the results of Yamashita et al. (1971) and Horowitz and Haurowitz (1959). The pH optimum for alpha-chymotrypsin was pH 7, in agreement with Tauber (1949, 1951), but in disagreement with Yamashita et al. (1971) who found an optimum pH of 5.5.

A 40% w/v substrate concentration was optimum for plastein synthesis for each enzyme (Fig. 2) which is in agreement with Fugimaki et al. (1977) and Hofsten and Lalasidis (1976).

Optimal plastein productivity was obtained after 4 hr of incubation at 37°C for each synthetic enzyme, with only small increases in plastein productivity after 24 hr (Fig. 3). Further incubation to 48 hr resulted in slight decreases in plastein formation, possibly due to enzymatic hydrolysis and/or disassociation of the plastein complex. Yamashita et al. (1970) and Fugimaki et al. (1977) suggested that a
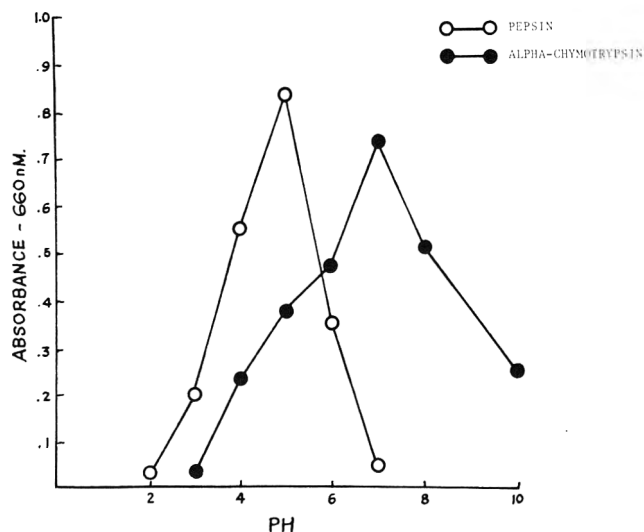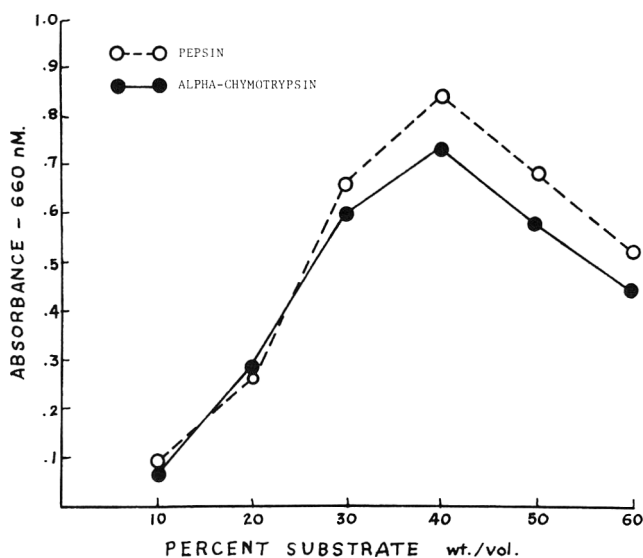
24-hr incubation period permitted the plastein chain to become assembled in a complete manner. Therefore, a 24-hr incubation was adopted as a working parameter for this study. In all cases, such stable gels were formed that when the tubes were inverted, the contents did not flow out; however, the gels were thixotropic and became fluid when the tubes were vibrated or shaken vigorously. Thixotrophy was reversible only when the gels were freeze-dried, resuspended in buffer and incubated at 37°C for 4 hr.

The effect of the enzyme/substrate ratio upon the rate of plastein production is shown in Fig. 4. For each enzyme, a ratio of 1:50 yielded the greatest rate of plastein formation during the first 4 hrs of incubation. When the ratio was decreased to 1:100 and 1:200, correspondingly slower rates of plastein formation were found. Therefore, the rate of plastein formation appears to be dependent upon the amount of enzyme added. Fugimaki et al. (1977) suggested a 1:100 ratio on the basis of economics. Furthermore, since

Fig. 3—Effect of time on plastein production using pepsin and alpha-chymotrypsin. Enzyme/substrate ratio=1:100 w/w; substrate concentration=40% w/v; pH=5 for pepsin, pH=8 for alpha-chymotrypsin; temperature=37° C.

Fig. 4—Effect of enzyme/substrate ratio using pepsin and alpha-chymotrypsin upon the rate of plastein production. Substrate concentration=40% w/v; pH=5 for pepsin, pH=7 for alpha-chymotrypsin; temperature=37° C.

Fig. 5—Effect of the approximate degree of hydrolysis upon plastein production. Enzyme/substrate ratio=1:100 w/w; substrate concentration=40% w/v; pH=5 for pepsin, pH=7 for alpha-chymotrypsin; temperature=37° C; incubation=24 hr.

isolate with plastein productivity. These authors stated that the degree of hydrolysis and pH were the most important parameters controlling the plastein reaction and net plastein yield.

Yields of enzymatically induced plastein

The results in Table 4 indicate the percent yields of plastein for each enzyme, employing 10% TCA as the precipitating agent. Percent plastein yields of 33.8 and 27.3 were found for pepsin and alphachymotrypsin, respectively. When plastein was precipitated from the gel using ethanol at a final concentration of 50%, the percent plastein yields increased to 46 and 40.5 for pepsin and alpha-chymotrypsin, respectively, as indicated in Table 4. Ethanol-precipitated plastein did not contain lipid and contained approximately 1.0% ash (Table 5). In comparison, the 10% TCA precipitated plastein contained 70% protein and possessed ash content approaching 20%. The high ash content is due to residual TCA in the precipitable fraction and/or other salt complexes which, therefore, reduced the percent protein yield.

The results of this study compare favorably with those of Onoue and Riddle (1973) who found a 35% plastein yield using pepsin as the synthesis enzyme at pH 5 and peptic hydrolysates of whole fish waste as the substrate. The yields were based on percent plastein recovery using 50% ethanol as the precipitating agent. In a further study, Edwards and Shipe (1978) investigated plastein yields from peptic hydrolysates of a commercial egg albumin and found yields of 30% employing pepsin as the synthetic enzyme, 23% for alpha-chymotrypsin and 10% for papin. From the results of this study using flounder frame protein hydrolysates as substrate, pepsin was the most plastein productive enzyme.

CONCLUSIONS

CONDITIONS necessary for optimal plastein productivity from flounder frame protein hydrolysates were established. The following optima were found: pH 5 for pepsin and pH 7 for alpha-chymotrypsin; substrate concentration, 40%

there was no net increase in plastein formation after 24 hr incubation for each enzyme concentration, a 1:100 ratio was adopted for this research.

The effect of approximate degree of hydrolysis upon plastein synthesis is shown in Fig. 5. When the approximate degree of hydrolysis was less than 60% for each enzyme, the amount of turbidity, as the 10% TCA insoluble fraction, generated from the substrate and enzyme controls exceeded the amount of turbidity generated from the plastein sample. This suggests that hydrolysis was occurring in the plastein sample in place of synthesis. However, when the degree of hydrolysis exceeded 60% (i.e. after 8 hr incubation), the direction of the reaction shifted towards synthesis of plastein (i.e. the amount of 10% TCA insoluble fraction of plastein sample exceeded that of the substrate and enzyme control). Optimum synthesis was found with a 24 hr hydrolysate, corresponding to an approximate degree of hydrolysis of 80%. No increase in plastein formation was found in using the 48 hr hydrolysate.

Yamashita et al. (1970) found a similar pattern in assessing the influence of degree of hydrolysis of a soy protein

Table 4—Comparison of percent yield and percent plastein yields for plastein reaction products using 10% TCA and 50% ethanol as precipitating agents

Product	Amount (g)	% Protein	Protein (g)	% Yield[a]	% Plastein yield[b]
Pepsin Plastein (TCA)	4.10	71	2.91	47.0	33.8
Pepsin Plastein (ETOH)	3.40	94	2.81	46.1	46.1
Alpha-chymotrypsin (TCA)	3.40	72.7	2.47	40.5	27.3
Alpha-chymotrypsin (ETOH)	2.71	91.2	2.47	40.5	40.5
Substrate Control[c] (TCA)	1.31	61.1	0.81	13.2	- - - -
Substrate Control[c] (ETOH)	<0.1	- - - -	- - - -	- - - -	- - - -

[a] % yields were calculated from a starting quantity of 10g hydrolysate at 61.1% protein representing 6.11g of protein.
[b] % plastein yield = % yield enzyme sample — % yield control
[c] Substrate control = 24 hr peptic hydrolysate

Table 5—Proximate analysis of freeze-dried peptic hydrolysates and plastein

	% Protein[a]	% Lipid[b]	% Ash	% Moisture
Peptic hydrolysate	61.1	2.6	27.1	8.5
Pepsin plastein-50% ethanol insoluble fraction	94.0	0.0	0.71	5.4
Alpha-chymotrypsin plastein-50% ethanol insoluble fraction	91.2	0.0	1.1	7.4
Pepsin plastein-10% TCA insoluble fraction	71.0	0.4	19.4	8.7
Alpha-chymotrypsin plastein-10% TCA insoluble fraction	72.7	0.4	18.9	8.9

[a] Kjeldahl nitrogen x 6.25
[b] Soxhlet extreaction with diethyl ether

w/v; incubation time, 24 hr; enzyme to substrate ratio, 1:100 w/w; approximate degree of hydrolysis, 80%. Percent plastein yields of 33.7 and 27.3 were found for the synthetic enzymes, pepsin and alpha-chymotrypsin, respectively, using 10% TCA. When plastein was precipitated by 50% ethanol, percent plastein yields were found to be 46 and 40.5 for pepsin and alpha-chymotrypsin. Protein values were found to exceed 90% for the ethanol insoluble plastein. Pepsin was the most plastein productive enzyme. Further studies involving functional, nutritional and physiochemical properties of flounder frame protein isolates and plastein will be reported.

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Strawberry Fruit Softening: The Potential Roles of Polyuronides and Hemicelluloses

D. J. HUBER

——————— ABSTRACT ———————

Polyuronides and hemicelluloses derived from ethanol powders or cell walls of strawberry (*Fragaria* x *ananassa*, Duch. Dover) receptacle tissue were examined to determine if these wall polymers might be involved in the softening of this fruit. Throughout maturation and ripening, total polyuronides increased on a per fruit basis although as a percentage of ethanol powder they remained constant. Gel-filtration chromatography confirmed that polyuronide solubility was not correlated with extensive enzymic hydrolysis, an observation consistent with the fact that D-galacturonanase (polygalacturonase) activity was not detected in strawberry fruit. The sugar composition of alkali-soluble wall polymers showed little change throughout development. However, changes occurred in the molecular weights of these polymers during ripening.

INTRODUCTION

IT IS WELL KNOWN that the softening which occurs in many fruit types during ripening involves D-galacturonases (polygalacturonases), the activity of which may ultimately result in a loss or solubilization and sometimes degradation of cell wall polyuronides (Huber, 1983b). In some fruit types the situation is far less clear. In apple fruit, the presence of only *exo* D-galacturonase (Bartley, 1978) fails to explain the release of polyuronides. Strawberry fruit also show increased levels of soluble polyuronides during softening (Woodward, 1972; Knee et al., 1977) yet the nature of this solubilization is unknown. Gizis (1964) reported that D-galacturonases were present in strawberry fruit and Woodward (1972) likewise suggested, based on measurements of viscosity of extracted polyuronides, that enzymic action was involved in polyuronide solubilization. Other workers (Neal, 1965; Barnes and Patchett, 1976) have been unsuccessful in attempts to find D-galacturonanases in strawberry fruit. Neal (1965) suggested that increased methylation might be responsible for polyuronide solubilization but other studies have shown that the degree of esterification of strawberry fruit polyuronides is low (14%) and does not change during development (Wade, 1964).

The aim of this study was to investigate the mechanism responsible for the increased polyuronide solubilization which accompanies strawberry softening. Additionally, a recent report of a possible involvement of hemicellulosic polymers in tomato softening (Huber, 1983a) prompted a similar investigation of their potential role in strawberry.

MATERIAL & METHODS

Plant material

Strawberry fruits *Fragaria* x *ananassa*, Duch. Dover) were harvested from plants grown at the University of Florida-IFAS Agricultural Experiment Station located at Dover, FL. Fruit was selected at five developmental stages; small green (average diameter ca. 1 cm), large green (average diameter ca. 2 cm), white (fruit showing depleted levels of chlorophyll), pink, and red (commercially ripe). Fruit

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were surface sterilized in 0.05% NaOCl and rinsed with distilled water. Following removal of surface blemishes, the fruit were quartered and stored in polyethylene bags at −20°C.

Preparation of ethanol powders

Fifty grams fruit in 200 mL 100% ethanol were homogenized for 4 min using a Sorvall Omnimixer. The homogenate was refluxed for 30 min in a boiling water bath and then stored overnight at −20°C. The suspension was filtered through Miracloth (BioChemical Corp., La Jolla, CA) and washed with 500 mL of 80% EtOH (v/v) and then 1L 100% ethanol. Ethanol was removed by aspiration and the powder air dried at 24°C.

Preparation of cell wall

One-hundred grams strawberry fruit in 400 mL Na-phosphate buffer (30 mm, pH 6.0) containing 20 mM Na_2EDTA were homogenized for 4 min in a Sorvall Omnimixer. The homogenate was heated for 20 min in a boiling water bath, filtered through Miracloth and washed with 1L each 100 mM NaCl and acetone followed by 0.5L chloroform-methanol (2:1, v/v). Following aspiration, cell wall was stored in a vacuum desiccator over phosphorous pentoxide.

Isolation of polyuronides and hemicelluloses

Soluble polyuronides were prepared by suspending ethanol powder (20 – 50 mg) in 1 – 2.5 mL Na-acetate buffer (40 mM, pH 5.0) containing 20 mM Na_2EDTA. After 6 hr, the suspension was filtered through Miracloth and washed with 2 mL of the extraction buffer. The combined filtrate and washing were either used directly (gel chromatography) or dialyzed (ion-exchange chromatography).

Hemicellulose B fractions were prepared as described by Huber (1983a). Briefly, 250 mg cell wall were placed in 7 mL 4N NaOH containing 9 mg mL^{-1} $NaBH_4$. After two successive 8 hr extractions, the combined filtrates were neutralized with acetic acid and dialyzed, in turn, against running tap water (12 hr), 10% methanol (24 hr) and distilled water (2 x 24 hr). This preparation was used directly or stored at −20°C.

Gel-filtration and ion-exchange chromatography

Gel-filtration of polyuronides was carried out on a bed (60 cm high, 1.5 cm wide) of Ultrogel AcA 24 (Bio-Rad Lab., Richmond, CA) packed in Na-acetate-acetic acid buffer (30 mM, pH 5.0) containing 10 mM Na_2EDTA and 50 mM NaCl. Approximately 4 mg polyuronide in 2 mL of the extraction buffer were applied to the column and gravity eluted at a flow rate of 12 ml $\cdot$ cm^{-2} $\cdot$ hr^{-1}. Half-mL aliquots of the 2 mL fractions were analyzed for acid-sugars (A_{520}) (galacturonic-acid equivalents) using the Blumenkrantz and Asboe-Hanson (1973) procedure. Ion-exchange chromatography of polyuronides was performed on a bed (15 cm high, 1.3 cm wide) of DEAE Sephadex (Sigma Chemical Co., St. Louis, MO) which had been equilibrated and packed in Na-phosphate buffer (15 mM, pH 6.8) containing 20 mM NaCl and 5 mM Na_2 EDTA. Polyuronides (8 mg), following dialysis against the equilibration buffer, were applied to the column and eluted with the phosphate buffer. After 60 mL were collected, the column was washed with a NaCl gradient generated from 150 mL phosphate buffer and 150 mL buffer containing 800 mM NaCl. Fractions of 5 mL were collected at a flow rate of 10 mL $\cdot$ cm^{-2} $\cdot$ hr. Acid sugar content (A_{520}) was measured using the procedure described (Blumenkrantz and Asboe-Hansen, 1973) and pentose content (A_{660}) using the orcinol (Dische, 1953).

Hemicelluloses (3 mg) were applied to a bed (60 cm high, 1.5 cm wide) of Ultrogel AcA 34 and eluted with Na-citrate-phosphate buffer (15 mM, pH 5.5) containing 100 mM NaCl. Fractions of 2

mL were collected and analyzed using the phenol-sulfuric acid (A_{490}) procedure (Hodge and Hofreitor, 1962).

Gas chromatography

Hemicellulose preparations or polyuronides recovered following ion-exchange chromatography were dialyzed against distilled water for 48 hr. A volume equivalent to 3 mg of these polymers was air dried in Reacti-Vials (Pierce Chemical Co., Rockford, IL) and hydrolyzed and acetylated as described by Loescher and Nevins (1972). The derivatized sugars were examined on a column packed with SP 2340 (Supel Co, Inc., Bellefonte, PA).

Measurement of total and soluble polyuronides

Total polyuronides were measured in essentially the manner described for cell wall by Ahmed and Labavitch (1977). To 5 – 7 mg ethanol powder was added 2.5 mL of chilled, concentrated H_2SO_4. After two successive 5 min intervals, 0.8 mL aliquots of distilled water were added. After a final 5 min period, 30 ml of distilled water were added. Acid sugars were measured as described above. Soluble polyuronides were measured by placing 20 mg powder in 7 mL of Na-acetate buffer (30 mM, pH 5.0) containing 10 mM Na_2EDTA. Over a 6 hr period at 23°C, aliquots were removed and filtered through glass fiber filter papers. The filtrates were examined for acid sugars.

Enzyme assays

Extracts for enzyme assays were prepared by homogenizing 25g strawberry fruit in 50 mL of Na-acetate buffer (40 mM, pH 5.0) containing 0.5% NaCl and 10 mM dithiothreitol. After 12 hr at 4°C, the homogenates were centrifuged at 10,000 x g and the supernatant dialyzed against the acetate buffer containing 100 mM NaCl. In other experiments, strawberry tissue (10g) along with 10g ripe avocado tissue or tipe tomato tissue were homogenized and enzyme preparations prepared as described above. Extracts were assayed for D-galacturonanase activity viscometrically (endo) using pectin or reductrometrically (exo) using polygalacturonic acid (2 mg mL^{-1}), both prepared in Na-acetate buffer (20 mM, pH 5.0) containing 100 mM NaCl. Enzyme extracts were utilized at an amount equal to 10% (v/v) of the substrate. Exoenzyme activity was also examined by gel filtration of reaction mixtures on a Bio-gel P-2 column.

RESULTS

Polyuronide analyses

The strawberry fruit is somewhat unusual in that it continues to increase in weight throughout development, including ripening. The data in Fig. 1 illustrate that this weight increase is accompanied by a marked increase in ethanol insoluble material, although on a per unit fresh weight
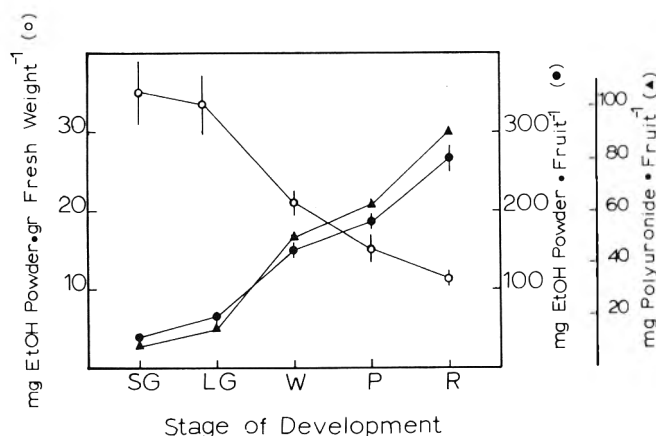
basis a large decrease is apparent. Total polyuronides as a percentage of ethanol powder remained constant throughout development, thus the data indicate continued synthesis of polyuronides during ripening (Fig. 1). The proportion of soluble polyuronides as a percentage of total polyuronides ranged from about 30% in undeveloped fruit to a high of 65% in ripe fruit, and the major trend of increasing soluble polymers occurred during ripening (white through red (Fig. 2).

Molecular size features of soluble polyuronides showed little consistent change at any stage during development (Fig. 3). Although some evidence for lower-molecular-size polymers was apparent in polyuronides from white and red fruit (Fig. 3B and C), the use of gels with higher exclusion limits (Agarose 1.5m) did not reveal further differences in the polyuronides. O'Beirne and van Buren (1983) have pointed out that the molecular size characteristics of apple fruit polyuronides may be explained, in part, by their tendency to form interpolymeric aggregates. Thus, it is possible that limited enzymic degradation occurred but was of insufficient magnitude to produce products which fractionated on Ultrogel AcA 34. In spite of these limitations, gel filtration has been quite useful at disclosing the products of in situ enzymic modification of polyuronides in tomato fruit (Huber, 1983a). Strawberry polyuronides following treatment with an enzyme extract from tomato fruit eluted similarly to polyuronides recovered from ripening tomato fruit (Fig. 3, C; Huber, 1983a). The similar elution behavior of the polyuronides from all stages of development examined is evidence that polyuronide degradation may not be a contributing factor in the increased solubilization of polyuronides during ripening. This observation was consistent with the apparent lack of either endo or exo D-galacturonanase at any stage of fruit development (data not shown). Examination of carbohydrates soluble in the ethanol homogenates revealed no polymeric or oligomeric uronides and only trace quantities of galacturonic acid (data not shown). A possible involvement of inhibitors of pectohydrolytic activity was investigated by cohomogenizing strawberry fruit with tomato or avocado fruit, both known to contain D-galacturonanases (Pressey, 1977). Assays of extracts so prepared exhibited no change in activity rela-

Fig. 1—Changes in ethanol insoluble powder and total polyuronides during strawberry fruit development. (○) mg ethanol insoluble powder per g fruit fresh weight, (●) mg powder per individual fruit, (▲) mg total polyuronide per individual fruit. Standard error bars are shown.
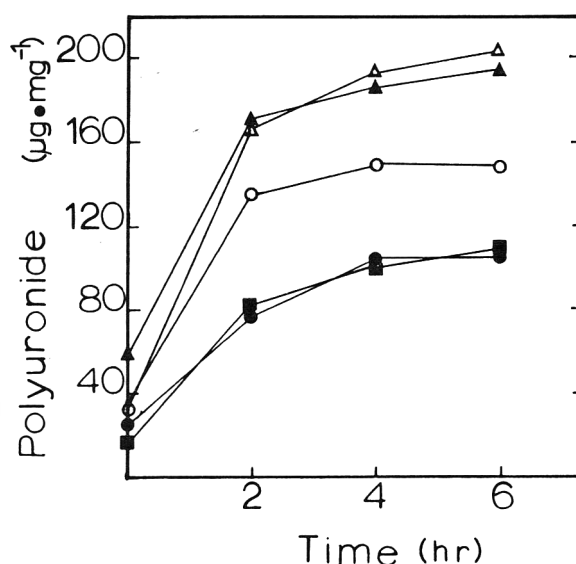
Fig. 2—Extraction of soluble polyuronides from ethanol insoluble powders. Units are given as μg galacturonic acid equivalents per mg powder (dry weight). (●) small green fruit, (■) large green fruit, (○) white fruit, (▲) pink fruit, (△) red fruit.

tive to tomato or avocado fruit extracts prepared in the absence of strawberry fruit. The evidence of course does not preclude the involvement of a highly specific inhibitor. The gel filtration studies and enzyme assays together would support the view that polyuronide solubilization in strawberry does not occur via hydrolysis of the rhamnogalacturonan backbone.

It is recognized that noncovalent interpolymeric associations of polyuronides are influenced by the presence of branches, typically composed of neutral sugars, and even more so by the presence of intrapolymeric insertions (i.e.

rhamnose). These factors could conceivably influence the solubility characteristics of these polysaccharides. Since it was possible that neutral polymers which are not structural components of polyuronides might be removed in the extraction procedure, prior to neutral sugar analysis, polyuronides were subjected to ion-exchange chromatography. Fig. 4 represents DEAE profiles of polyuronides from fruit at three selected stages of development. All five stages were examined and those presented are representative. Only small quantities of neutral sugar and no polyuronides were eluted with the starting buffer. Nearly all of the neutral sugars eluted in the gradient and were observed to comigrate with polyuronide. The neutral sugar components of the polyuronides are shown in Fig. 5. No major changes were appaent between small green and large green fruit. However, in fruit at the white stage, in essence representing the onset of ripening, neutral sugars arabinose, galactose and rhamnose increased. Further increases occurred as ripening progressed. While not the predominant neutral sugar, rhamnose exhibited the most dramatic increase (fivefold), resulting in an overall decrease in the molar ratio of galacturonic acid to rhamnose from 120 at the large green stage to 20 at the ripe stage.
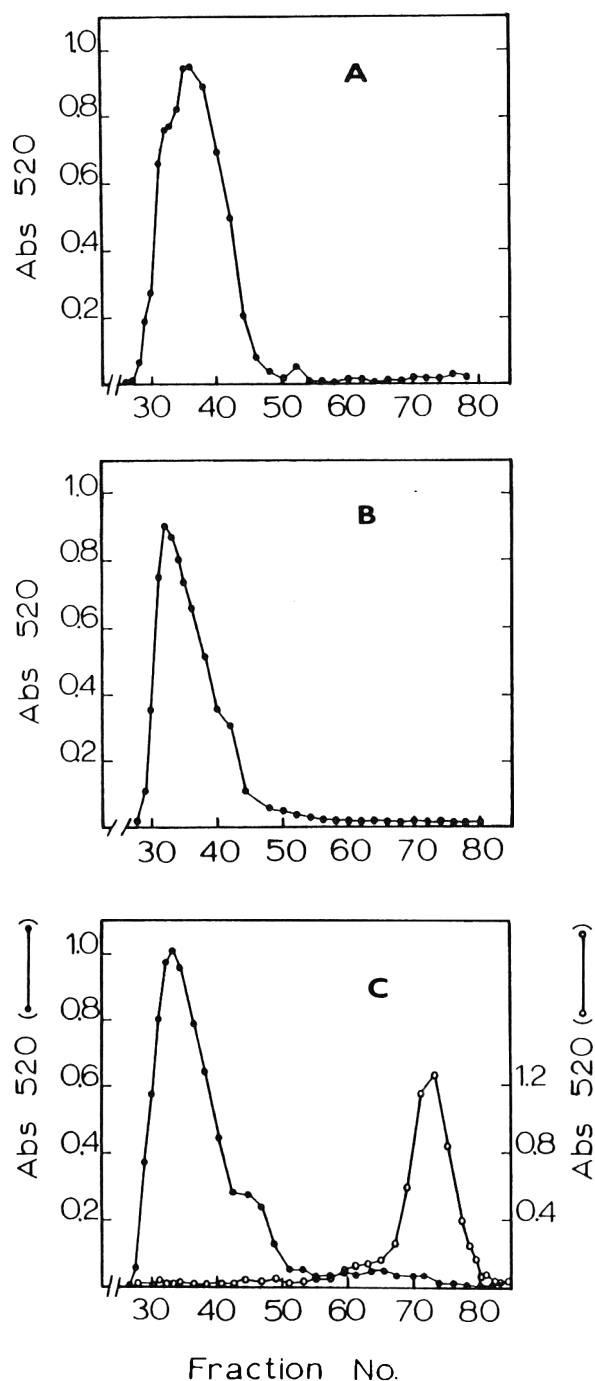
Fig. 3—Ultrogel AcA 34 chromatography of soluble strawberry polyuronides. Profiles represent polyuronides from (A) small green, (B) white and (C) red fruit. Open circle (o) profile in C was obtained by treating polyuronides from red fruit with an enzyme extract from ripe tomato fruit.
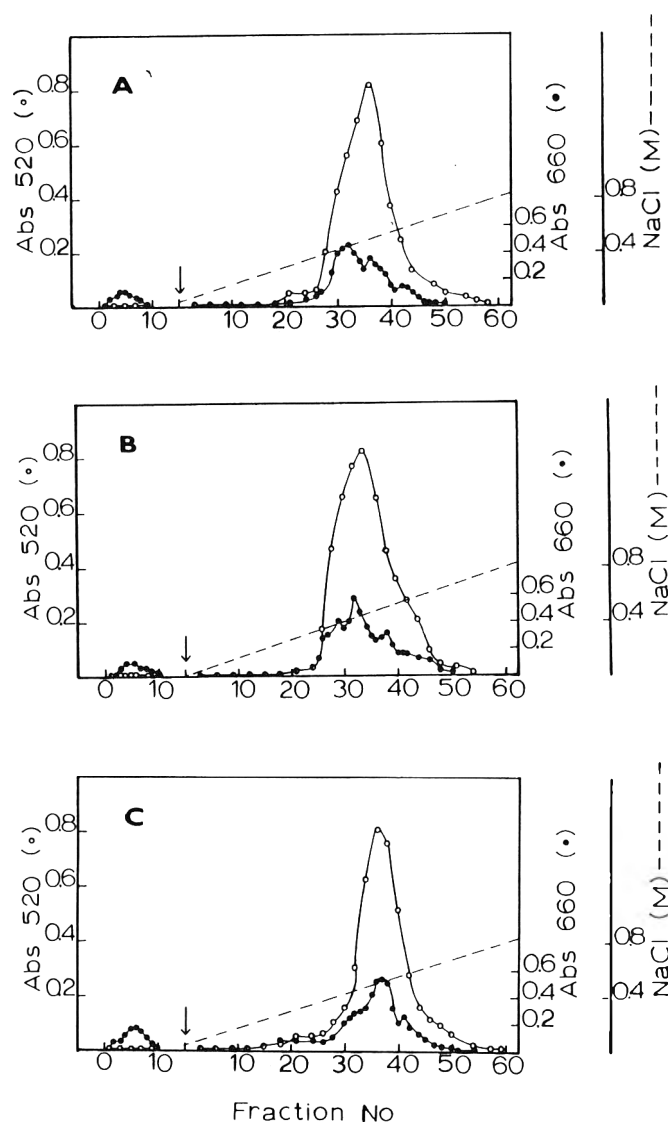
Fig. 4—DEAE Sephadex chromatography of soluble strawberry polyuronides. Profiles represent polyuronides from (A) small green, (B) white, and (C) red fruit. Vertical arrow indicates initiation of the gradient.

Hemicellulose analyses

Studies of tomato fruit hemicelluloses (Huber, 1983a) revealed that during ripening these wall polymers exhibited alterations resulting in an overall increase in the proportion of low molecular weight polymers. This constituted the first report of changes in the molecular weight of hemicelluloses in ripening fruit and emphasized the fact that compositional analysis, the classical approach to studying cell wall neutral sugar changes during ripening, may not always be an appropriate method for detecting wall metabolism affecting fruit texture. For this reason, hemicelluloses from strawberry fruit were similarly examined. The gel filtration profiles in Fig. 6 show very evident changes in molecular-weight distribution of the hemicelluloses during strawberry fruit development. Hemicelluloses from small green and large green fruit yielded similar elution patterns on Ultrogel (only large green shown in Fig), showing a consistence predominance of high-molecular weight polymers. Changes were first evident in fruit at the onset of ripening (white stage) and more alteration was apparent as ripening proceeded (Fig. 6D and E). The hemicelluloses from red (ripe) fruit showed a very evident trend toward a predominance of low-molecular-weight polymers. Compositional analysis of the hemicelluloses revealed the presence of arabinose, galactose, xylose and glucose as major components. Little change in neutral sugar composition accompanied the molecular weight changes observed (Table 1) with the exception of minor but consistent decreases in arabinose and galactose. Polyuronide content of hemicellulose preparations never exceeded 5%.

DISCUSSION

THE STRAWBERRY RECEPTACLE, unlike most fruit types, continues to accumulate volume and mass throughout development. Along with these increases, net polyuronide synthesis is maintained even during ripening. Woodward (1972) reported a similar trend for strawberry polyuronides but found that some loss in total polyuronides occurred during the final stages of ripening. In the present study, the transition of berries from pink to red (ripe) was marked by a 30% increase in total polyuronide per fruit.
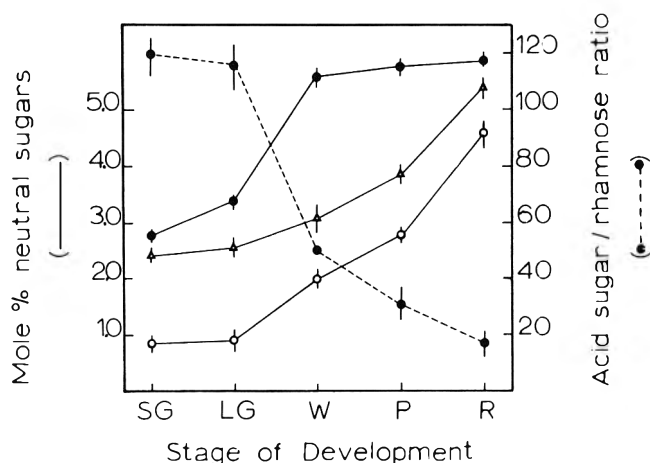
Fig. 5—Neutral sugar composition of polyuronides recovered following ion-exchange chromatography. Neutral sugars were examined employing gas chromatography. (○) Rhamnose, (△) galactose and (●) arabinose are expressed as mole percent of galacturonic acid equivalents. Standard error bars are shown and are based on results obtained from three samples.
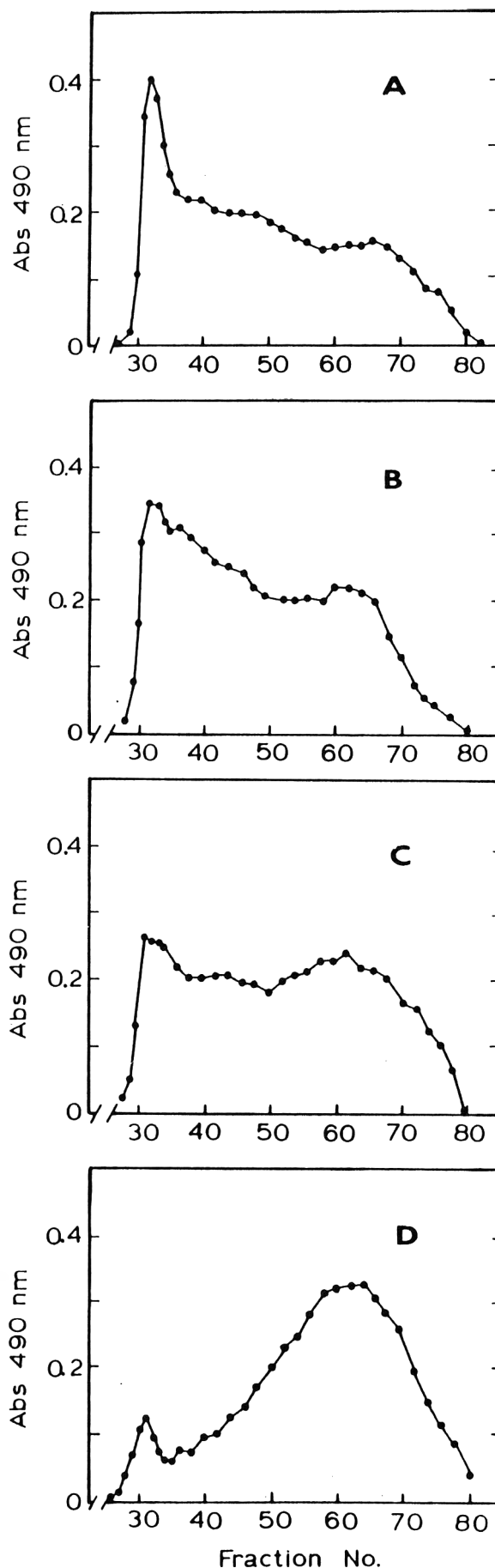
Fig. 6—Ultrogel AcA 34 chromatagraphy of strawberry fruit hemicelluloses. Profiles represent hemicelluloses from (A) large green, (B) white, (C) pink and (D) red fruit.

The levels of soluble polyuronides exhibited little change during early fruit development but at the onset of and throughout ripening (white through red) they increased markedly. In a number of fruit types, increased levels of soluble polyuronides are closely correlated with the appearance of pectohydrolytic enzymes (Huber, 1983b), and evidence that polyuronide degradation occurs has been provided by reports of molecular weight decreases in polyuronides solubilized (Pressey et al., 1971; Huber, 1983a)' Polyuronides solubilized in strawberry were not accompanied by molecular weight changes indicative of enzymic modification, at least as could be discernible using Ultrogel AcA 34. The fact that neither endo- nor exo-D-galacturonanase could be detected in strawberry fruit was further evidence that the role of enzymic hydrolysis in the increased solubility of polyuronides was questionable. Enzyme inhibitors were apparently not a factor since strawberry extracts had no influence on the activity of either tomato or avocado fruit enzymes. If D-galacturonanases are involved in polyuronide solubilization in strawberry, their action must clearly be limited. This possibility in turn would pose the question of what factor might restrict a more extensive degradation by such an enzyme. Polyuronides from strawberry fruit were rapidly degraded to low molecular weight polymers and oligouronides by an endo-D-galacturonanase prepared from tomato fruit. The most apparent change occurred in the levels of neutral sugars associated with soluble polyuronides. Polymeric arabinose and galactose are well known to frequently be associated with polyuronides (Darvill et al., 1980). In strawberry, the proportions of these neutral sugars associated with polyuronides remained constant throughout early development and increased during ripening, at which time soluble polyuronides increased. Increased solubilization or loss during ripening of cell wall galactose and arabinose have been reported for many fruit types (Knee, 1975; Yamaki et al., 1979; Gross and Wallner, 1979; Ahmed and Labavitch, 1980); however, the origin of these sugars and the mechanism responsible for their loss is not clear for all cases. In ripening pear (Ahmed and Labavitch, 1980), solubilized arabinose was found to be a structural component of a branched polyuronide (pectin arabinan). Gross and Wallner (1979) found that the release of wall neutral sugars (primarily galactose) occurred in the tomato ripening mutant, *rin*, which showed little increase in soluble polyuronides. In strawberry, the evidence indicates that the solubilized neutral sugars arabinose and galactose are present primarily as structural components of polyuronides. Other changes included an increase in the proportion of rhamnose. This increase is perhaps related to the increases observed for arabinose and galactose since arabinans and galactans are apparently linked to polyuronides via the rhamosyl moieties (McNeil et al., 1980). As a component of the galacturonan backbone, it is conceivable that rhamnose could have a profound influence on the confirmation of the polymer and, just as important, on the inter-

polymeric associations of these polysaccharides. Analyses have revealed that rhamnose insertions result in a marked kinking of the parent polymer, minimizing the frequency of interaction of any two adjacent chains (Grant et al., 1973; Morris et al., 1977). One consequence might be a weakening of interchain associations, which for maximum stability require prolonged, alligned associations possibly involving calcium.

The results of the present study indicate that the increased levels of soluble polyuronides are due largely to the synthesis of a modified, more freely soluble form during ripening. The increased proportions of arabinose, galactose and rhamnose might all be contributing factors. Investigators working with tomato (Knegt et al., 1975; Sawamura et al., 1978) and apple (Knee, 1978) have also proposed a relationship between polyuronide synthesis and solubility during ripening yet how the two events might be related is largely unknown. The presence of pectolytic enzymes in these fruits precludes a definitive statement on the mechanism, enzymic or otherwise, of polyuronide solubilization. It might be argued for strawberry fruit that softening results not from polyuronide hydrolysis but rather from the inability of the tissue to maintain structural integrity as a result of the addition to the wall of less tightly bound, more freely soluble polyuronides. An additional possibility which can not be discounted is that the tissue during ripening exhibits a loss in its capacity to convert the newly synthesized polyuronides to a more tightly bound form characteristic of less developed fruit.

A recent study (Huber, 1983a) of ripening tomato fruit revealed that in addition to polyuronide solubilization and degradation, hemicelluloses were also altered, showing an increasing proportion of low-molecular weight ($<40,000$) polymers during ripening. Similar changes were observed in strawberry, and again these changes were temporally related to softening and ripening. No major change was observed in the composition of alkali-soluble polysaccharides (Table 1) however the trend was clearly one of decreasing molecular weight. Preliminary studies have provided some evidence that the mechanism responsible for these changes in strawberry may be enzymatic. In tomato fruit, no hemicellulase activity was apparent (Huber, 1983) but it was clear that the modifications occurred independently of polyuronide degradation. The results with strawberry again emphasize the limitations in the use of compositional analyses to study the wall metabolism of ripening.

The results of these studies indicate that both polyuronides and hemicelluloses may be important in influencing the texture of strawberry fruit. The fact that the changes observed were not apparent during early fruit growth is evidence that they were not related to growth per se but rather were more likely involved in the texture changes associated with ripening.

Table 1—Sugar composition of strawberry fruit hemicelluloses (Values given represent composition on a mol-% basis)

| Sugar | Stage of development | | | | |
	Small green	Large green	White	Pink	Red
Arabinose	20.2	24.7	21.7	19.5	16.9
Xylose	29.6	25.6	25.8	26.1	29.4
Galactose	19.5	19.5	19.5	19.8	18.0
Glucose	25.2	25.4	28.0	27.2	28.6
Mannose	3.1	2.0	2.3	3.4	3.4
Rhamnose	0.6	0.7	0.5	0.9	0.6
Fucose	1.9	2.0	2.1	2.1	2.2

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SUMMARY

MASS AVERAGE VELOCITY, $\bar{u}$, can be calculated from the total volumetric flow rate, (it may also be obtained experimentally).

Eq. (17) allows the ratio of actual kinetic energy to that calculated for plug flow for a H-B fluid to be calculated analytically. Also, this value may be estimated using Fig. 1.

Eq. (2), (14) and (18) allow the KE of a H-B fluid to be calculated analytically for flow in circular tubes.

Kinetic energy correction factor [Eq. (18)] is obtained by using Fig. 2.

NOMENCLATURE

C $= \tau_0 / \tau_w$
E_f = energy loss due to friction, J/kg
k = consistency coefficient, Pa s^n
KE = average kinetic energy per unit mass of fluid, J/kg
L = length of tube, m
n = flow behavior index, dimensionless
r = radial distance, m
P = pressure, Pa
Q = volumetric flow rate, m^3/s
R = radius of tube, m
u = local velocity, m/s
$\bar{u}$ = mass average or bulk velocity, m/s
W = work term in the mechanical energy balance, J/kg
α = kinetic energy correction factor, dimensionless

Z = potential energy, J/kg
δP = pressure drop over a pipe of length L, Pa
$\dot{\gamma}$ = rate of shear, s^{-1}
ρ = fluid density, kg/m^3
τ = shear stress, Pa
τ_0 = yield stress, Pa
τ_w = shear stress at the wall, Pa

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A Study of the Forces Involved in the Incorporation of L-Methionine into Soy Protein by the One-Step Plastein-Like Process

S. R. NOAR and W. F. SHIPE

──────── ABSTRACT ────────

Ultrafiltration was used to study the forces involved in the incorporation of methionine into soy protein. Various solvents were used to determine the extent of noncovalent binding. When analyzed with 0.2M citric acid, 60% of the methionine was incorporated into the soy protein. However, when analyzed with 8M Guanidine HCl and 3M KSCN the incorporation was only 20 and 13%, respectively. This reduction in percent incorporation of methionine by protein denaturants indicates that it was not incorporated by covalent bonds. Incorporation is probably due to hydrophobic attraction of the side chain of methionine with apolar regions in the protein.

INTRODUCTION

MUCH WORK has been done on the plastein reaction to enrich a protein with an amino acid. After finding that free L-methionine did not react when trying to add methionine to soy protein, it was determined that the ethyl ester was the most successful amino acid derivative for incorporation (Yamashita et al., 1971). Soy protein plastein has been significantly enriched with methionine using L-methionine ethyl ester (Yamashita et al., 1971; Arai et al., 1974).

While the plastein reaction is believed by some to involve covalent incorporation (Yamashita et al., 1971, 1972; Monti and Jost 1979), the results are by no means conclusive. There are others who feel that covalent forces do not play a role in the plastein reaction and instead believe that the incorporation is due to noncovalent forces (v. Hofsten and Lalasidis, 1976). A close relationship has been found between the hydrophobicity of the amino acid ethyl ester and the initial velocity of the incorporation (Aso et al., 1977). Yamashita et al. (1975a) and Arai et al. (1976) concluded that incorporation of the amino acid ethyl esters depends primarily on the hydrophobicity of the amino acid side chains. Aso et al. (1977) found that the longer the chain length of the esterifying alcohol, and thus the greater the hydrophobicity, the greater the incorporation of the amino acid esters.

One possible explanation for the importance of hydrophobicity is that the incorporation of hydrophobic amino acids produces an insoluble complex which readily precipitates from the reaction system. This is considered to serve as a driving force for the reaction to proceed efficiently (Yamashita et al., 1975b). In fact, it has been found that plastein products have more hydrophobic amino acids than the original substrates (Aso et al., 1973).

The feasibility of using the plastein reaction for protein enrichment is limited to some extent by the costs and complexity of the two-step process. Consequently, Yamashita et al. (1979) developed a set of reaction conditions to combine the two steps and facilitate the incorporation of methionine into soy protein in one step. The product from this one-step process cannot be called a plastein. Though the plastein reaction is believed by some to involve covalent incorporation of amino acids into protein, the

mechanism of the one-step process has not been studied. Therefore this research was undertaken to study the extent of hydrophobic and electrostatic forces in incorporation by the one-step process.

MATERIALS & METHODS

SOY PROTEIN, Promine F, an isoelectric precipitate was obtained from Central Soya (Fort Wayne, IN). Papain, L-methionine, L-cysteine, L-methionine ethyl ester, guanidine HCl, and potassium thiocyanate (KSCN) were obtained from Sigma Chemical Co. (St. Louis, MO). Citric acid and urea were obtained from Mallinckrodt (St. Louis, MO).

Guanidine HCl, KSCN, citric acid and urea were used to prepare solutions for use in the ultrafiltration step. The particular compound and concentration used are specified in each table.

S^{35}-methionine was a gift from Dr. Volcker, Cornell Univ.

Preparaton of substrate

The soy protein was shaken with a 100-fold amount of 0.1N NaOH for 2 hr at room temperature. A small amount of insoluble substance was removed by centrifugation. The isoelectric reprecipitate (pH 4.5) was obtained and dialyzed against running water at 5°C for 3 days. The product was then freeze-dried.

Preparation of S^{35}-L-methionine ethyl ester

Fifty microcuries (μCi) of S^{35}-L-methionine were diluted with 0.5g nonradioactive L-methionine and esterified by the method of Hiskey and Harpold (1967).

The reaction products were dissolved in benzene and recrystallized as needles by adding petroleum ether. This was repeated until a single spot, with an R_f equal to that of purchased L-methionine ethyl ester, was obtained by thin-layer chromatography using cellulose powder coated plates and separated with a 80:20:4 solution of methanol-water-pyridine (Wollenmuller, 1962).

Enzymatic treatment

The procedure of Yamashita et al. (1979) was followed. One gram of the alkali-denatured, isoelectric precipitated, soy protein isolate (SPI), 0.05g methionine ethyl ester (nonradioactive), 0.01g papain, 1 mM L-cysteine and the required amount of NaOH solution were mixed to give a 20% SPI concentration at pH 10. Then 5 μL of S^{35}-L-methionine ethyl ester (25 μCi/mL) were added, the flask was flushed with nitrogen and capped.

After incubating for 12 hr at 37°C, 10 mL 0.1N NaOH was added and the sample allowed to stand for 5 hr at room temperature. Samples receiving the above enzymatic treatment are referred to as treated samples. The controls were handled in the same way except that the enzyme was omitted.

Ultrafiltration

Ten milliliters of the sample were added to an Amicon TCF10 ultrafiltration cell with a Nucleopore Type C (500 MW cutoff) membrane. Ninety milliliters of the desired solution were added and the sample was filtered under 35 PSI nitrogen. The first 10 mL filtrate were discarded and the next 10 mL were collected.

A 1 mL aliquot of the filtrate and retentate were assayed for S^{35} using a Beckman Spectronic 100-LSC liquid scintillation counter. The results are expressed in counts per minute (cpm).

Calculation of percent incorporation of methionine

The calculation is as follows:

$$Y - W = S \tag{1}$$

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$[S/(S + X) \times (100\%)] = \%$ of added methionine that is bound   (2)

where X = total amount of unbound added methionine in 99.5 mL (1g of SPI dissolved in each solution accounted for 0.5 mL of the total volume). Y = total methionine (ie. both free and bound) left in the retentate. W = free methionine in the retentate since the concentration (per milliliter) of free methionine in the retentate is in equilibrium with the free methionine in the filtrate. S = bound methionine.

RESULTS

THE RESULTS from a series of trials analyzed with a Type C (500 MW cutoff) membrane using 0.2M citrate (pH 2.2) as the ultrafiltration solution show that the enzymatically treated samples contained an average of 60% incorporated methionine while the controls had an average incorporation of 17%. The incorporation in the controls was probably due to the apolar association of the methionine with the unhydrolyzed protein. The hydrophobic side chain of the methionine might form apolar bonds with the limited amount of exposed hydrophobic regions on the soy protein.

Both 0.2M citrate and 5M guanidine HCl had little effect on disrupting the incorporation of methionine into the protein (Table 1). By contrast, 8M guanidine HCl greatly reduced the incorporation of methionine. Preliminary data revealed that urea (8M) also had little effect in reducing the incorporation into the protein.

Even though 5M guanidine HCl and 8M urea should be sufficient to disrupt hydrophobic forces in most systems (Haschemeyer and Haschemeyer, 1973), they were not adequate in this case. Since 8M guanidine HCl reduced the percent incorporation of methionine, it is postulated that the incorporation was due to hydrophobic forces.

Another compound that has a very destabilizing effect on protein conformation is KSCN (Haschemeyer and Haschemeyer, 1973). Table 2 shows the effects of increasing concentrations of KSCN on incorporation of methionine. The controls show that increasing the concentration of KSCN decreased the percent incorporation of methionine. Presumably this is due to the disruption of apolar bonds since these forces are probably the only ones involved in the nonenzymatic incorporation. In the treated samples, increasing the level of KSCN reduced the percent incorporation down to 14% in the case of 3M KSCN. In both cases it is concluded that charge-charge interactions are not responsible for the incorporation because 1M KSCN should be sufficient to eliminate such interactions.

DISCUSSION

WHILE THE ASSOCIATION of methionine with the protein in enzymatically treated samples apparently is more stable than the controls, it does not appear to be a covalent linkage since it can be disrupted by the appropriate concentration of guanidine HCl and KSCN.

Hydrophobic bonding, hydrogen bonding, or charge-charge interactions may be responsible for the noncovalent incorporation of methionine. According to a study by von

Hippel and Wong (1965), guanidine HCl can act effectively in disrupting both hydrophobic and hydrogen bonds. Neutral salts, such as KSCN, act by breaking up charge-charge interactions and by disrupting hydrophobic forces. The disruption of hydrophobic forces is the only effect that both compounds have in common.

Therefore it is concluded that the incorporation of methionine by the one-step process is due to the formation of hydrophobic bonds. These results cannot be compared with the plastein reaction due to the uncertainty about whether the plastein reaction and the one-step process involve the same mechanism. The fact that the one-step process results in a soft mass of protein whereas the plastein reaction results in a gel adds to this uncertainty.

The fact that KSCN has a greater effect than guanidine HCl and a high concentration of guanidine HCl was required probably reflected the unique characteristic of this particular protein system. It is generally known that every protein and protenacious system is affected differently and to different degrees by various physical and chemical factors. Also each denaturant will give different net results depending on how it alters the local water structure (von Hippel and Wong, 1965).

The role of the enzyme has not been established. Since hydrophobic bonding is a factor, then the increased incorporation following enzymatic treatment is probably due to the hydrolysis of the protein which exposes more hydrophobic regions. This would allow more apolar bonding of methionine. When the protein was first hydrolyzed and the methionine ethyl ester added after hydrolysis, it was found that there was little difference between the prehydrolyzed sample and the control (i.e. 16 and 17%, respectively). This shows the need for the methionine ethyl ester to be present while the enzyme is acting. This is probably because the protein will seek a more energetically stable configuration and refold to hide the newly exposed hydrophobic regions from the agueous phase and thus from the methionine ethyl ester added after incubation.

From this latter evidence, and the fact that the enzymatically induced incorporation was much more stable than the control, it is postulated that the enzymatically incorporated methionine is associated with interior (and thus protected) hydrophobic regions. The non-enzymatically incorporated methionine and the methionine ethyl ester added after incubation do not have access to these interior regions. Instead these incorporations are due to association with the limited amounts of exposed hydrophobic regions on the protein.

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Table 1—Comparison of the effect of citric acid and guanidine HCl on the percent incorporation of methionine into soy protein[a]

	Solvent media		
Sample[b]	Citric acid	5M Guanidine HCl	8M Guanidine HCl
1	64	62	21
2	61	68	19
3	66	53	19
4	54	55	22
Control	17	14	—

[a] Data given in terms of percentage of added methionine that is still bound after solvent treatment.
[b] Values are single determinations for different samples.

Table 2—Effect of concentration of potassium thiocyanate on the percent incorporation of methionine into soy protein[a]

	Concentration of KSCN (M)				
Sample	0.0	0.5	1.0	2.0	3.0
Control	64	37	28	8	—
Treated[b]	72(2)	47(2)	34(2)	27(1)	14(5)
Range	69-75	44-49	33-35	27	11-18

[a] Data given in terms of percentage of added methionine that is still bound after solvent treatment.
[b] No. in parentheses indicates number of replicates.

Canned Dry Bean Quality as Influenced by
High Temperature Short Time (HIST) Steam Blanching

S. R. DRAKE and B. K. KINMAN

──────────── ABSTRACT ────────────

High temperature short time (HTST) steam blanched dry beans had greater drained weights and shear values than water blanched dry beans. Moisture content of dry beans was greater after water blanching. Subjective grade was closely related to drained weights and shear values, and HTST steam blanched dry beans had better subjective grades than water blanched dry beans. Water blanched dry beans were lighter in color than HTST steam blanched dry beans. As length of HTST steam blanch was increased, Agtron color of the beans darkened. High quality canned dry beans can be produced with HTST steam blanching with energy and time savings, but differences between water and HTST steam blanching and canning quality is highly dependent on cultivar and length of HTST steam blanch.

INTRODUCTION

BLANCHING of vegetables consumes large amounts of energy and is a costly operation. Chhinnan et al. (1980) reported that blanching of spinach consumed 34% of the total energy required to process the product. Of this 34% only 31% was required to blanch the product whereas 69% of the energy was lost. Rose et al., (1981) concluded that water blanchers are more energy efficient than steam blanchers. Efficient blanching units minimize loss of uncondensed steam. To increase energy utilization, steam loss at the blancher must be reduced. Steam losses can be reduced by enclosing the blanch unit and adding water or mechanical seals when possible.

The blanching of a product prior to canning removes foreign material, improves color, aids in filling the can, and in the development of a desirable texture (Cain, 1950; Crafts, 1944). Prior to blanching, dry beans for processing are commonly soaked overnight (12 – 14 hr) at ambient temperature (Junek et al., 1980). Several researchers have indicated that shorter soaking times are sufficient for dry beans for canning (Nordstrom and Sistrunk, 1977; Neely and Sistrunk, 1979). Research on the comparison of water and steam blanching on the processing of dry beans and their subsequent quality is available. Pinto beans not blanched or steam blanched had greater drained weights and firmer texture than water blanched beans (Davis, 1976; Nordstrom and Sistrunk, 1977). Nordstrom and Sistrunk (1979) reported that steam blanched dry beans were firmer in texture with greater drained weights than water blanched beans, depending on cultivars used. Davis et al., (1980) found that blanch method and post-blanch treatment had significant effects on drained weights, shear values, and percent splits between dry bean cultivars. Cultivar and growing location (Quenzer et al., 1978) influence the color and texture of canned pinto beans. Shear values of processed dry beans are more reliable quality indices than water imbibition. Daoud et al. (1977) and Nordstrom and Sistrunk (1979) determined that water blanched dry beans retained less nutrients than steam blanched beans. Sevilla

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and Luh (1974) observed that steam blanching leaches less of the soluble components of beans than water blanching. McCurdy et al. (1983) determined that steam blanching produced processed dry peas with lower shear values, greater drained weights, and greater soluble solids in the brine than water blanched peas. Junek et al. (1980) found that canned dry bean quality can vary depending on storage time prior to processing.

This study was conducted to compare the quality of high temperature short time (HTST) steam blanched dry beans with water blanched dry beans, and to determine quality differences that may occur between dry bean cultivars.

MATERIALS & METHODS

DRY BEANS (*Phaseolus vulgaris* L.) were obtained from the USDA/ARS dry bean program at the Irrigated Agriculture Research & Extension Center, Prosser, WA. Three cultivars (Sanilac, NW63, and Viva) and three breeding lines (W34, GH206, and GH590) from the 1982 crop season were used. Each cultivar and breeding line was divided into 12 separate lots of 4 kg each, to permit three replicates for four separate treatments (a control and three HTST blanching times, 40, 60, and 80 sec). The control treatment for each cultivar was soaked 12 hr in distilled water at room temperature, drained, and splits and skins removed. Control beans were then water blanched at 88°C for 3 min and cooled in tap water.

Beans to be HTST blanched were presoaked at 74°C for either 10, 15, or 25 min. The time of presoaking was dependent upon a previously determined point of imbibition (Viva and W34 10 min; Sanilac, NW63, and GH206, 15 min; and GH590 25 min). After presoak, 4 kg of beans and an equal volume of water were immediately placed in a laboratory model (Key Technology, Inc.) Thermo Flo™ Food Processor (high pressure steam blancher) operating at 80 psi. The beans were blanched in the vessel for 40, 60, or 80 sec. At the end of the blanch cycle the steam pressure was released immediately and the beans were cooled in tap water. Blanched beans were packed in polyethylene bags, placed in an insulated cooler, transported to IAREC (Prosser, WA) kept in a walk-in refrigerator overnight, and canned the following day.

Two-hundred grams of blanched beans were placed in 303 x 406 cans, a 60 grain NaCl tablet added and the beans covered with distilled water at 93.3°C, allowing for a 6.4 mm headspace. Canned beans were sealed with an atmospheric closure, processed for 27 min at 118°C, and cooled in tap water.

After 30 days storage at ambient temperature, the processed beans were evaluated for moisture, drained weight, shear force, color and subjective quality; and the brine for turbidity. Drained weights and moisture were determined by AOAC procedures (1975). A Food Technology Corp., Texture Test System model TG-4, equipped with a multiple blade CS-2 cell was used to determine shear values. Shear values are reported as the amount of force in Newtons required to shear 100g of product. Color of the beans was measured with an Agtron Model 500A reflectance spectrophotometer using the blue mode (436 μm) for white beans and the red model (640 μm) for dark beans. Brine turbidity was determined on the white beans only using a Hach Turbidimeter and reported in Nephelometric Turbidity Units (NTU). The processed beans were graded subjectively for integrity and wholeness on a scale of 1 to 5, where 5 = excellent integrity or wholeness, 3 = fair integrity, and 1 = no integrity. Five individuals involved in dry bean research were used as subjective graders. The analysis of variance used cultivars (three levels) as the main plot, and blanch treatment (four levels) as the subplots. Analysis of variance was conducted separately for white (Sanilac, W34, GH590) and dark beans (NW63, Viva, GH206).

There was no consistent interaction between treatments so this was not considered in the text.

RESULTS & DISCUSSION

CULTIVAR AND BLANCH TREATMENT have an influence on quality attributes of canned dry beans (Tables 1 and 2). The white bean W34 contained more moisture after blanching and prior to thermal processing than GH590 or Sanilac, which contained the least moisture after blanching (Table 1). Drained weights were greater for GH590 than either W34 or Sanilac, which were similar. Brine was more turbid from cans of GH590 than from cans of Sanilac or W34 and turbidity of the brine was higher for Sanilac than W34. Agtron color (blue) was greatest for W34 and least for GH590 with Sanilac displaying an intermediate color. Shear values of GH590 were greater and the subjective grade lower than that for either W34 or Sanilac. Shear values for Sanilac and W34 were similar, but the subjective grade of W34 was greater than that of Sanilac. Although W34 was not as firm as Sanilac it had better integrity and wholeness.

Moisture after blanching was greater for the dark bean cultivar Viva than either GH206 or NW63 (Table 2). Drained weights and shear values were less for Viva than either GH206 or NW63. GH206 beans contained less moisture after blanching, and had greater drained weights and shear values than either Viva or NW63. The moisture content, drained weight, and shear values for NW63 were in-termediate between Viva and GH206. The Agtron color (red) and subjective grade was greater for GH206 than that for either Viva or NW63, which were similar.

Water blanched dry beans contained more moisture after blanching than any HTST blanch treatment except for Sanilac, GH590 and GH206 HTST steam blanched at 80 secs (Tables 1 and 2). Drained weights were greater for HTST steam blanched beans than water blanched beans, but as time of steam blanching was increased, differences were minimized. Water blanching produced lower drained weights than steam blanching, which is expected. The reported changes in drained weights observed due to water vs. steam blanching varied tremendously. McCurdy et al. (1983) noted differences in drained weights of less than 9% with dry peas whereas Nordstrom and Sistrunk (1979) reported differences in drained weights of less than 2% with dry beans between water and steam blanching. In this study the differences in drained weights between water and steam blanching were as high as 16% for white beans and 22% for dark beans. As the time of steam blanching was increased the differences in drained weights between water and steam blanching was reduced. The difference in drained weights of white beans between water and HTST steam blanched for 80 sec were similar for W34 and GH590, and less than 1% for Sanilac, but for dark beans the differences varied from 11% for GH206 to 16% for Viva.

The turbidity of the brine (Table 1) for water blanched white dry beans was greater than HTST blanch treatments,

Table 1—Mean quality attributes of canned dry white beans as influenced by cultivar and blanch treatment

Treatment		Moisture after blanching (%)	Drained wt (g)	Brine turbidity (NTU)	Agtron blue	Shear (Newtons)	Subjective grade
Cultivar	Blanch						
Sanilac	Water	50.1 a[a]	308 d	25 b	47 a	445 a	3.3 b
	Steam 40 sec	41.9 c	370 a	29 ab	41 b	350 b	3.6 b
	Steam 60 sec	46.7 b	316 c	32 a	40 b	420 a	4.3 a
	Steam 80 sec	49.5 a	330 b	32 a	35 c	421 a	4.3 a
W34	Water	56.6 a	315 c	29 a	48 a	349 d	3.3 c
	Steam 40 sec	50.0 c	355 a	27 ab	47 a	389 c	3.7 c
	Steam 60 sec	52.8 bc	327 b	25 b	39 b	429 a	4.6 b
	Steam 80 sec	53.3 b	314 c	21 c	42 b	460 a	5.0 a
GH590	Water	53.0 a	335 c	44 a	35 a	458 a	3.2 d
	Steam 40 sec	46.5 c	394 a	37 b	35 a	414 b	2.6 c
	Steam 60 sec	48.6 b	354 b	30 c	32 b	434 ab	3.8 b
	Steam 80 sec	50.5 ab	332 c	28 c	31 b	468 a	4.6 a

[a] Means in a column within a cultivar followed by different letters are significantly different (P < 0.05).

Table 2—Mean quality attributes of canned dry dark beans as influenced by cultivar and blanch treatment

Treatment		Moisture after blanching (%)	Drained wt (g)	Agtron red	Shear (Newtons)	Subjective grade
Cultivar	Blanch					
NW 63	Water	51.3 a[a]	327 b	37 a	422 c	2.9 d
	Steam 40 sec	41.3 c	381 a	28 b	439 bc	4.3 a
	Steam 60 sec	45.8 b	371 a	28 b	456 b	3.9 b
	Steam 80 sec	43.3 c	383 a	28 b	553 a	3.4 c
Viva	Water	53.9 a	301 c	27 b	391 c	3.3 b
	Steam 40 sec	42.0 c	385 a	34 a	438 b	3.9 a
	Steam 60 sec	45.4 b	361 b	32 a	456 ab	3.7 a
	Steam 80 sec	47.5 b	359 b	32 a	481 a	3.9 a
GH206	Water	41.8 a	343 c	42 a	708 a	5.0 a
	Steam 40 sec	37.8 b	427 a	42 a	516 c	2.7 c
	Steam 60 sec	42.4 a	398 b	40 ab	595 b	4.2 b
	Steam 80 sec	41.7 a	388 b	36 b	682 a	5.0 a

[a] Means in a column within a cultivar followed by different letters are significantly different (P < 0.05).

except for Sanilac beans where the turbidity was less for water blanched beans. Turbidity decreased as time of HTST steam blanch was increased for W34 and GH590 beans, but the opposite was true for Sanilac beans. Water blanched dry beans had a lighter color (higher Agrtron blue) than any HTST blanched dry beans. Agtron blue values were reduced as the time for HTST blanching increased from 40 to 80 sec, resulting in darker colored beans. Agtron blue values were similar for 60 or 80 sec HTST blanching, except for Sanilac beans. Agtron red values of beans (Table 2) darkened as time of HTST blanch was increased for NW63 and GH206, but Viva beans tended to lighten in color.

Water blanched dry beans W34, NW63, and Viva, (Tables 1 and 2) had lower shear values than dry beans HTST blanched. Shear values for Sanilac, GH590 and GH206 water blanched were similar to HTST blanched 60 or 80 sec. There was no significant difference in shear values between 60 and 80 sec HTST blanched beans except for NW63 and GH206. HTST beans blanched for 40 sec had the lowest shear values. Subject grade increased as time of HTST was increased over the subject grade for water blanched beans, except for GH206. The subjective grade for GH206 was similar for water blanched or HTST steam blanched for 80 sec. GH206 beans HTST blanched 40 sec had a very low subjective score.

Based on the literature available (Davis, 1976; Nordstrom and Sistrunk, 1977), there is little doubt that steam blanched beans have a greater drained weight than water blanched beans. Drained weights are dependent upon the dry bean cultivar (Davis, 1980; Nordstrom and Sistrunk, 1979; Quenzer et al., 1978). Drained weights in this study confirm that HTST blanched beans have a greater drained weight than water blanched beans and are dependent upon the cultivar in question. Shear values or texture difference between water and HTST blanched beans depends upon the time beans are exposed to the steam and moisture content after blanching. Water blanched beans were greater in moisture values than HTST blanched beans prior to thermal processing and in all cases these high moisture value beans had lower drained weights and shear values than HTST blanched beans depending upon cultivar. This agrees with a previous study (McCurdy et al., 1983) on dry peas where steam blanched dry peas have lower shear values than water blanched dry peas.

The subjective grade for canned dry beans in this study was significantly ($P < 0.05$) greater for HTST blanched than water blanched beans but this was dependent upon the time of steam blanching and cultivar. A high subjective grade was also directly related to low drained weights and high shear values. The turbidity of the brine was dependent upon cultivar and blanch treatment. Water blanched beans had a less turbid brine than HTST blanched beans.

Agtron blue and red color of beans was dependent upon cultivar and blanch. The color of water blanched beans was lighter than HTST blanched beans. As the time of HTST blanching was increased from 40 to 80 sec, the white beans tended to darken. The dark colored beans also darkened as blanch time was increased, except for 'Viva', which tended to lighten in color.

CONCLUSIONS

HTST STEAM BLANCHED BEANS have greater drained weight and shear values than water blanched beans depending upon length of the blanch and bean cultivar. Moisture content is greater in water blanched beans after blanching, and additional moisture in the beans prior to canning results in less bean solids in the can to absorb water, reduced drained weights and shear values. Subjective grade is closely related to drained weight and shear values and HTST blanched beans had greater subjective grades than water blanched beans. Agtron color was lighter for water blanched beans than HTST blanched beans and as length of the HTST blanch was increased, Agtron color of beans darkened.

High quality canned dry beans can be produced with HTST steam blanching with possible subsequent energy and time savings, but cultivar and time of the blanch must be determined in advance.

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Utilization of Distillers' Spent Grain in Extrusion Processed Doughs

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————————————ABSTRACT————————————

The potential of utilizing distillers' spent grain (DSG) as a component of a dough system for the manufacture of puff-extruded type products was studied. The DSG was incorporated at various concentrations with four flours, functioning as the supporting matrix, and subjected to extrusion processing. Evaluation of the extrudates indicated the DSG had a large effect on the quality of the final product in terms of structure and flavor. Both subjective and objective evaluations of the DSG-flour extrudates indicate the DSG can be incorporated into the extrusion doughs up to a concentration of 20% with highly acceptable products being manufactured, but levels above this resulted in inferior quality products.

INTRODUCTION

DISTILLERS' SPENT GRAIN (DSG) is a major by-product produced from the fermentation residue after the distillation of the alcohol. Corn and other grains are commonly used for this fermentation process due to their high starch content and availability. During the fermentation process, the starch content is greatly reduced by the yeast and converted to alcohol, carbon dioxide, and other fermentation products. This reduction in starch results in as much as a threefold concentration of the other grain components such as protein, fat, ash, and fiber.

The use of this potentially valuable fermentation by-product as an animal feed supplement has been reviewed extensively by many researchers (Chen et al., 1977, Distillers Feed Research Council, 1981; Harms et al., 1969; Matterson et al., 1966; Schabinger and Knodt, 1948). In all studies reviewed DSG demonstrated positive effects on animal growth, weight gain, and milk production. Due to the high nutritional content of DSG, food scientists have recently become interested in its possible food application.

The potential of using the nutritional characteristics of DSG to supplement selected food commodities is of interest because incorporating DSG into these food systems offers a possibility of another protein source for the human diet while utilizing an unconventional grain. There is minimal information available concerning the use of DSG in food products. Limited success has been achieved in the flour-dough area according to available reports. In one study, DSG was evaluated as a possible bread ingredient replacing a percentage of the wheat flour (Tsen et al., 1983). Another study investigated the possibility of using DSG as a cookie supplement (Tsen et al., 1982). In both studies, DSG was found to be a suitable supplement in the dough at levels of 10–15%. The objective of this study was to determine if the DSG could be a suitable supplement in a dough system undergoing puff extrusion. The objective of the sensory evaluation portion of this study was to evaluate the effect of DSG concentration on flavor.

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MATERIALS & METHODS

THE CORN DISTILLERS' SPENT GRAIN — the coarse, fibrous portion which is separated by screens and dried (Distillers' Feed Research Council, 1981) — was supplied by the Distillers Research Council. The corn DSG was ground in a Willey Mill through a 1 mm screen. The ground DSG flour was incorporated at levels from 0–40% in 10% increments into several flours commonly used for the manufacture of low density extruded products. The commonly used flours included soft red spring wheat, yellow degerminated corn, and commercially available medium grain rice and potato; for each dough formula the DSG flour was incorporated with one of the other flours.

The DSG flour formula was mixed in a Hobart, A-200-FD, mixer for 20 min. The pH of the formula was adjusted to 7.0 and the moisture content adjusted to 15%; these adjustments were facilitated by adding the determined crystalline sodium hydroxide to water and then spraying the solution into the dough during the final 10 min of mixing.

The formulated dough was extruded in a Bonnot 2-¼ inch (5.72 cm) cooking extruder through a 2.5 mm die. The temperature and screw speed were selected from results of preliminary experiments. The screw was operated at a speed of 200 rpm, and the temperature of each head was as follows: Head #1, 10°C; Head #2, ambient; Head #3, 121°C; Head #4, 138°C. The extrudate was cut into 3.81 cm pieces and dried in a Carrier forced air dehydrator for 30 min at 66°C to a final product moisture content of 2%. The dried extrudate was evaluated for expansion ratio values. The expansion ratio values were based on the average of 20 samples selected randomly and were expressed as the ratio of the diameter of the extrudate to the diameter of the die orifice.

The dried extrudates were also evaluated by an experienced sensory panel consisting of ten graduate students; the samples were presented in a random order to each member. The panel evaluated the extruded product for flavor using a scale where a score of 10 was an excellent rating, and a score of one was a poor rating. The taste panel results were evaluated statistically using Duncan's Multiple Range Test (SAS, 1979). All evaluations were performed in a sensory evaluation laboratory with partitioned booths and artificial sunlight lighting.

RESULTS & DISCUSSION

Effect of DSG concentration on degree of expansion

To evaluate the effect of DSG on the degree of expansion of the extrudate, the four flours were supplemented with the DSG at levels of 0%, 10%, 20%, 30%, and 40%, and extruded. The DSG had a significant effect on the degree of expansion for the extrudate as shown by the large reduction of the expansion ratio values for the extruded products as the DSG concentration increased (Fig. 1).

The DSG-rice doughs and the DSG-potato doughs responded in a similar manner in terms of the expansion ratio. For the DSG-rice dough and the DSG-potato dough formulations, the expansion ratio values at the 0% DSG concentration were very low with values of 2.0 and 1.4, respectively; extrusion of these two doughs was extremely difficult. When the DSG was incorporated at the 10% and 20% concentration levels, the doughs produced extrudates with extremely high expansion ratio values. These DSG-rice doughs yielded expansion ratio values of 5.6, for the 10% DSG concentration level and 5.0 for the 20% DSG concentration level. The DSG-potato doughs had very similar expansion ratio values with values of 6.0 and 5.5 at

Fig. 1—Effect of DSG on the expansion ratio values for each DSG-supporting flour extrudate.

Table 1—Taste panel flavor evaluation results for the DSG-supporting flour extrudate as influenced by the concentration of DSG in the formulation[a]

Conc of DSG (%)	Flour-DSG formulation						
	Corn X̄		Rice X̄		Potato X̄		Wheat X̄
0	6.0	B	3.4	C	1.1	D	4.3 B
10	7.6	A	6.0	A	6.5	A	6.5 A
20	6.2	B	6.3	A	5.2	B	6.2 A
30	4.2	C	4.1	B	3.6	C	3.7 BC
40	3.5	C	3.5	BC	2.8	C	2.9 C

[a] Average scores with different capital letters are significantly different (p < 0.05)

of DSG the majority of the starch has been removed during the fermentation process. At the low DSG concentration levels, the dough could support this virtually non-functioning component, but at the higher levels, this component caused a collapse of the extrudate matrix. For the most part, the poor functionality of the DSG grain coupled with the high fat content resulted in a collapse of the extrudate matrix at the high DSG concentration yielding inferior quality products.

Effect of DSG concentration on taste panel evaluations

The taste panel results were similar for all DSG-flour extrudates (Table 1). The 10% DSG concentration level was found by the taste panelists to be very acceptable with scores ranging from 6.0–7.6. As with the expansion ratio values, as the concentration of DSG in the dough increased, the flavor scores decreased. Flavor scores were poor at the 30% and 40% DSG concentration levels. In all cases the scores for the flour without any DSG. The panelists noted a mild astringent, grainy flavor at the 10% DSG level which was found to be acceptable, but as the DSG concentration increased, the characteristic DSG flavor became overpowering and unacceptable.

CONCLUSIONS

IT IS APPARENT from this study that DSG can be easily and quite acceptably incorporated into rice, potato, wheat, and corn flour doughs. At the 10% DSG level, the expansion ratio values and the taste panel scores were found to be very acceptable. The 20% DSG level, while not as good as the 10% level, was also acceptable. These findings indicate that DSG can be used as a flour component for the production of puff-food products and subsequently utilize a valuable by-product from the distilling industry.

the DSG concentration levels of 10% and 20% respectively. As the DSG concentration was increased in the dough to levels of 30% and 40%, the expansion ratio values dropped drastically and resulted in highly dense brown products. The expansion ratio values for the 30% and 40% DSG concentration levels for the DSG-rice dough were 2.7 and 2.0 and for the DSG-potato doughs were 3.5 and 3.0, respectively.

The DSG-wheat doughs and DSG-corn doughs also responded similarly overall, but initially responded differently than the DSG-rice and DSG-potato doughs. At the 0% DSG concentration level, the doughs produced their highest expansion ratio values with values of 5.1 for both the DSG-corn and DSG-wheat extrudates. Gradual declines were noted for both doughs as the concentration of DSG in the dough was increased. The DSG-wheat doughs had expansion ratio values of 4.6 at the 10% DSG concentration level and 4.0 at the 20% DSG concentration level; the DSG-corn doughs declined from 4.8 for the 10% DSG level and 4.7 for the 20% DSG level. As with the DSG-rice and DSG-potato doughs, the DSG-wheat and DSG-corn doughs yielded drastically reduced expansion ratio values at the 30% and 40% DSG concentration level. The DSG wheat doughs produced expansion ratio values of 2.7 and 2.2 for the 30% and 40% DSG concentration levels respectively; the DSG-corn doughs had very similar results.

Data from previous studies indicate that the fat content of the DSG has a major effect on the expansion ratio values. The fat content of the rice and potato flour is low in comparison to the corn and wheat flour (0.1%, 0.4%, 2.6%, 1.0%, 8.0% for the potato, rice, corn, wheat, and DSG flour, respectively). The fat level of the rice and potato flour is not sufficient to facilitate proper dough development during extrusion; this is supported by the low expansion ratio values and the production of high density burnt products. As the DSG was added to these flours, the expansion ratio values improved significantly; the resulting fat content of these doughs was adequate for proper extrusion. For all the doughs, as the DSG was incorporated at the 30% and 40% levels, the fat content was too high permitting too much slippage during extrusion and subsequent low expansion ratio values. In general, the high fat content of the DSG produced negative effects at the high DSG incorporation levels.

The other major effect studied was the functionality of the DSG grain. In puff extrusion the degree of expansion, and thus quality of the extrudate, is greatly dependent on the ability of the starch to form a stable matrix; in the case

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Effect of Postharvest Storage and Ripening of Apples on the Sensory Quality of Processed Applesauce

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─────── ABSTRACT ───────

Pre-processing low temperature storage of apples tended to increase the perceived sweetness and decrease sourness (tartness) of finished applesauce. Storage had no significant effect on the sheen and fruitiness of sauce and no effect on the overall acceptability ratings of flavor and texture. Grain size decreased on storage. Depending upon cultivar, color was adversely affected by storage. High temperature ripening tended to darken sauce but did not adversely affect the overall acceptability ratings of color and flavor. Ripening increased the fruitiness and decreased the grain size with some interactions between ripening and storage on grain size. Sweetness and sourness were not significantly affected by ripening treatments. The overall rating of texture was decreased with ripening.

INTRODUCTION

APPLES FOR SAUCE are seldom processed immediately following removal from the tree. This may be due to a number of circumstances such as a wish to permit further ripening of the fruit to make sauce of a certain desired property, the necessity of using up previously harvested fruit to avoid excessive spoilage, or simply the need to lengthen the processing season. This can be done under a variety of conditions. Seldom, if ever, can processors afford to utilize the sophisticated but much more costly holding conditions of controlled atmosphere storage. In any case, any condition of holding necessarily involved increased ripeness of the fruit. The higher the temperature, the more rapid the ripening process occurs. For simplification, in this paper holding fruit at $0°C$ will be referred to as "storage," and at $18°C$ will be refered to as "ripening."

Though most processors make applesauce from stored and/or ripened fruit, few studies have investigated the combined storage and ripening effects on applesauce quality. Wiley and Toldby (1960) studied factors affecting the quality of canned applesauce including storage time. They found that color and texture improved up to at least 50% of the storage life of the cultivars. Their findings indicated that flavor was not affected by storage. Lee et al. (1965) studied physical and biochemical factors influencing applesauce quality and drew no conclusions on the effect of storage time. Objective measurements of graininess were studied in relation to storage treatments. Lanza and Kramer (1966) reported that longer storage time yielded smaller particle size. They also noted significant interactions between cultivar and storage. The effect on applesauce quality of ripening, alone or in combination with storage treatments has not been adequately addressed.

The objective of this study was to determine the effect of storage and ripening treatments of apples on the quality of finished sauce. Both the combined and individual effects of these treatments were included in the design. Specific sensory characteristics were measured as well as overall preferences.

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MATERIALS & METHODS

THIS EXPERIMENT was based on a three factorial design. Each factor in the design had three levels: cultivar (Cortland, Idared, Monroe) storage (0, 5, 10 wk) and ripening (0, 5, 10 days). Fruit were harvested from Experiment Station orchards at the commercially acceptable harvest date for each cultivar. Samples were randomized and subsamples from each cultivar were ripened immediately and again after 5 and 10 wk at 0°C storage.

Following each period of storage and ripening, raw product analysis was conducted on each sample. These analyses included fruit firmness, soluble solids, and titratable acidity.

The balance of the fruit, approximately 25 kg per treatment combination, was processed under standard conditions into sauce according to the method of LaBelle et al. (1960) with the modification that the impeller speed of the finisher was decreased to 800 rpm instead of 1000 rpm and soluble solids of the finished product was decreased to 16°Brix ± 1°.

Processing data were taken. Total yield and finisher waste were calculated. Sauce samples were analyzed for sugar/acid ratio (Winkler, 1932), hue values calculated from Hunter Colorimeter measurements (Little, 1975) and average particle size (Kimball and Kertesz, 1952).

Canned sauce was stored at approximately 21°C for 2 months prior to sensory analysis. Samples were submitted to an experienced panel consisting of eight persons for a Quantitative Descriptive Analysis (QDA) (Stone et al., 1974). Panelists were selected on their ability to discriminate and reproduce results from difference tests. The first three descriptors used were quality ratings of the sauce and the last six were measurements of various sensory characteristics (McLellan et al., 1984). Each attribute on the ballot was interpreted using a typical QDA nonnumeric linear scale with a low-medium-high or similar notation (Table 1). Numerical interpretation was handled by digitizing the data by computer to produce an answer reflecting a 0-10 scale. Testing was carried out in a sensory evaluation laboratory, in partitioned booths under standardized daylight conditions. Samples placed in souffle cups were submitted in groups of three and coded using a three digit random number. Water was present for rinsing. Panelists were instructed to evaluate each product presented, individually, starting with overall quality ratings, visual appearance, aroma, tastes, and mouthfeel. Each panelist received triplicate samples of all 27 treatment combinations in a completely random order.

An analysis of variance was conducted on the QDA responses. Significant differences between means were tested for using Tukey's "Honestly Significant Difference" test which utilizes a t-like statistic based on the distribution of the *Studentized Range*. A minimum significant difference (MSD) value was derived to compare the differences between means. Differences were noted as being significant at $a = 0.05$ or less.

RESULTS & DISCUSSION

Physical evaluation

Evaluation of raw product, processing properties and resulting quality of the sauce were generally similar to that previously reported (LaBelle et al, 1960; Wiley and Toldby, 1960). Salient features of these analyses are presented in Table 2. They will not be discussed in detail. Briefly, results of the analysis of raw product at the time of processing for all cultivars and all treatments were consistent with what would be expected of progressively increasing ripeness with the passage of time. All treatments resulted in softening in all cultivars, Cortland and Monroe softening

more than did Idared. Sugar/acid ratios increased with all treatments with all cultivars.

Processing data for the manufacture of sauce generally indicated decreasing finisher waste more or less proportional to the decrease in fruit firmness with fruit held at both temperatures. Total yield of sauce was generally decreased by ripening when conducted immediately after harvest, and following storage. This was evident in the amounts of water added to the product to attain the desired consistency.

Physical characterization of the resulting sauce indicated, a progressive cultivar-dependent change in color due to treatment. Monroe and Idared cultivars became browner with preprocessing storage and Cortland lightened with storage. Hue values did not significantly vary due to storage. Ripening produced a lighter sauce as was indicated by the mean response for Hunter L value: 83.1, 83.7, 85.8, for 0, 5, 10 days ripening, respectively.

There was also a pronounced trend towards a smaller average particle size in all sauce when ripened, and only slightly less when stored. The effect of the 10 days ripening on average particle size of the sauce for each cultivar at each storage treatment is presented in Fig. 1. A steady decline in particle size was seen in all cultivars until 10 wk storage and ten days ripening. Although Corland continued to decrease in average particle size after an additional five weeks of storage, Idared and Monroe increased in average particle size. For these two cultivars, these findings run counter to general trends previously reported (Lanza and Kramer, 1967).

Table 1—Sensory attributes included on the QDA ballot with appropriate scale notations

Attribute	QDA scale notation		
Overall Quality Ratings			
Color	poor	—	good
Flabor	poor	—	good
Texture	poor	—	good
Specific Sensory Measurements			
Color	white — yellow	—	brown
Sheen	dull	—	glossy
Fruity Apple Aroma	low — medium	—	high
Sweetness	low — medium	—	high
Sourness (Tartness)	low — medium	—	high
Grain type	fine	—	coarse

Sensory evaluation

Overall quality ratings (poor - good) for the three sensory attributes (color, flavor and texture) were each affected differently by the experimental conditions (Table 3). The overall flavor ratings were not significantly different due to any combination of cultivar, storage, or ripening. This supports the findings of Wiley and Toldby (1960).

Table 2—Raw product and sauce processing data

Weeks in storage (0°C)	0			5			10		
Days ripened (18°C)	0	5	10	0	5	10	0	5	10
Raw product analysis									
Cortland									
Firmness (1bf)	15.0	13.0	11.3	12.7	8.8	8.0	10.3	8.4	7.9
Total acid (%)[a]	0.51	0.51	0.44	0.44	0.47	0.36	0.37	0.35	0.33
Soluble solids (%)	13.2	14.6	14.1	15.1	14.5	14.2	14.6	14.2	14.9
Monroe									
Firmness (1bf)	16.7	14.3	11.2	14.5	11.6	10.6	11.6	10.5	10.0
Total acid (%)[a]	0.56	0.52	0.45	0.47	0.46	0.39	0.42	0.35	0.34
Soluble solids (%)	16.2	16.6	15.4	16.7	16.5	16.2	16.7	15.5	15.4
Idared									
Firmness (1bf)	15.9	15.6	13.3	15.1	13.2	11.0	12.9	12.0	9.8
Total acid (%)[a]	0.47	0.46	0.43	0.47	0.43	0.37	0.39	0.41	0.35
Soluble solids (%)	13.9	13.2	13.4	14.4	13.8	13.1	13.4	13.2	12.6
Processing data									
Cortland									
Finisher waste (%)	5.3	4.5	3.5	5.0	2.8	4.7	3.9	2.1	1.9
Water added (%)[b]	53.5	9.0	2.1	17.3	6.4	0.3	14.2	8.4	0.0
Yield (%)[c]	86.5	85.9	72.2	88.8	83.3	75.2	88.0	85.7	77.9
Monroe									
Finisher waste (%)	4.2	4.0	3.3	4.4	3.4	2.9	4.3	3.7	3.3
Water added (%)[b]	50.9	25.0	21.8	44.2	25.7	25.5	22.8	19.8	23.4
Yield (%)[c]	95.9	96.2	94.5	105.4	100.8	97.8	88.8	90.9	82.2
Idared									
Finisher waste (%)	4.9	4.8	4.2	6.4	4.3	3.6	5.0	3.6	3.8
Water added (%)[b]	45.8	27.4	19.6	35.9	23.2	9.0	17.7	20.0	15.4
Yield (%)[c]	79.8	77.9	81.6	95.1	98.4	85.5	84.6	85.2	85.6

[a] Expressed as % malic acid
[b] To 4.5 Bostwick (66°C) consistency
[c] Final sauce/raw product apples
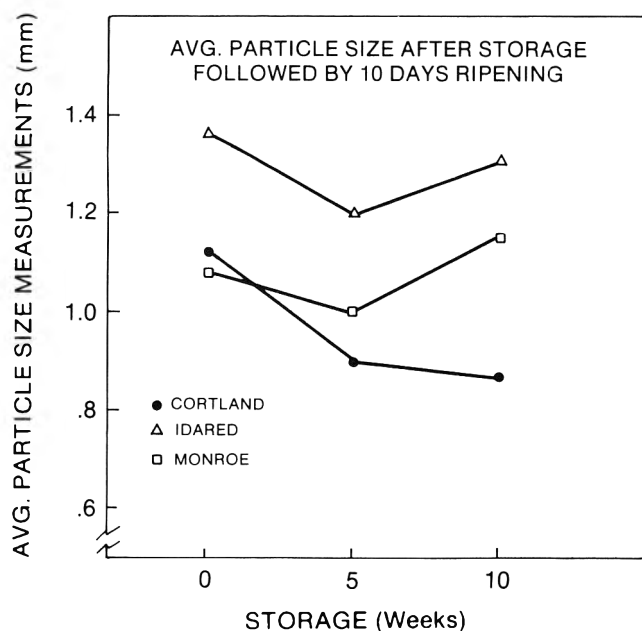
Fig. 1—Average particle size of applesauce after storage treatments followed by 10 days ripening.

Fig. 2—Mean QDA color response for applesauce by cultivar and storage treatments.

Table 3—Significance of main factors and treatment combinations based on F statistics from analysis of variance for each descriptor

Descriptor	Cultivar (C)	Storage (S)	Ripening (R)	SxR	CxS	CxR
Overall Quality Ratings						
Color	* *	* *	NS	*	* *	NS
Flavor	NS	NS	NS	NS	NS	NS
Texture	* *	NS	* *	NS	NS	* *
Specific Sensory Measurements						
Color	* *	* *	*	NS	* *	NS
Sheen	NS	NS	NS	NS	NS	NS
Fruity Aroma	* *	NS	*	NS	NS	NS
Sweetness	NS	* *	NS	NS	NS	NS
Sourness	*	* *	NS	NS	NS	NS
Grain Type	* *	* *	* *	* *	*	* *

* Significant at $a = 0.05$ level
** Significant at $a = 0.01$ level
NS Not statistically significant

Fig. 3—Mean QDA sensory responses for both sweetness and sourness in applesauce by storage treatments.

Rating scores of color were not changed by ripening treatments. However, cultivar x storage interactions were significant. This result generally reflected an improvement in the color rating scores for Monroe during storage. The ratings of the other two cultivars decreased slightly during storage. Wiley and Toldby (1960) also found a trend of improved color. The cultivar x storage interaction, however, indicated the importance of cultivar to cultivar variation and storage effects were found to be very cultivar dependent in terms of color rating. Scores for texture were not altered significantly due to storage effects but ripening x cultivar interactions were significant. Scores for texture tended to decrease during ripening for all three cultivars. Cortland applesauce decreased in texture rating dramatically during ripening.

Of the six individual sensory attributed measured, in addition to the quality ratings, sheen (dull-glossy) was not significantly affected by the treatment combinations (Table 3).

Ripening treatments and cultivar x storage interactions were significant for sensory color (white-yellow-brown)

measurements. Ripening tended to produce a browner applesauce. This was evident in Hunter hue values as previously discussed. This change did not affect overall color acceptability ratings as previously described. Panelists indicated Monroe and Idared cultivars became browner with pre-processing storage treatments but Cortland lightened with storage (Fig. 2).

Fruity aroma response was significantly different due to cultivars (Table 3). Ripening tended to increase fruitiness, however storage had no significant effect.

Perceived sweetness of the applesauce was not significantly affected by cultivar or ripening treatments (Table 3). Storage produced an insignificant drop and then an increase in sweetness. Sourness response was equal and opposite that seen for sweetness due to storage treatments (Fig. 3). Al-
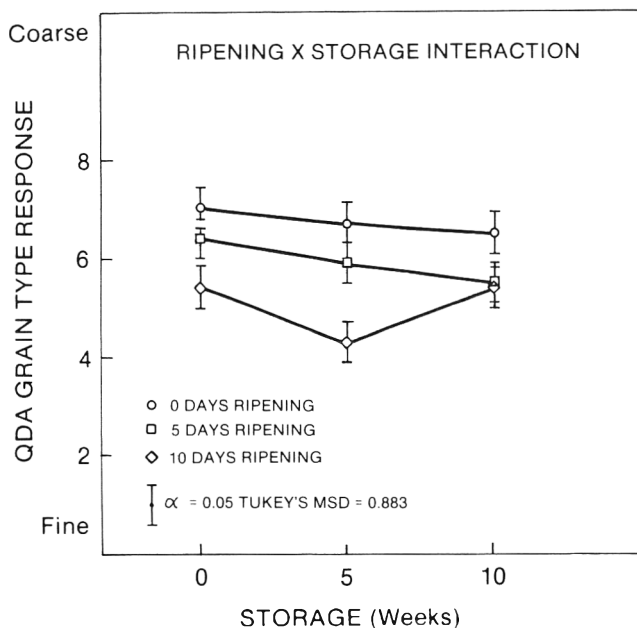
Fig. 4—Mean QDA grain type response for applesauce by storage and ripening treatments.

though sugar-acid ratios significantly increased during ripening treatments. Sourness was significantly different due to cultivar.

The panelists perception of grain type (fine-coarse) was found to vary significantly due to cultivar, storage, and ripening treatments (Table 3). The significance of cultivars is well supported in the literature (Lanza and Kramer, 1967; Mohr, 1973). Both storage and ripening tended to decrease grain size in the sauce. Cultivar x storage and cultivar x ripening interactions were significant primarily due to the extreme sensitivity of Cortland apples. Storage and ripening treatments produced a sharp drop in perceived particle size in this cultivar. The storage x ripening interaction was also significant. At 10 wk storage followed by 10 days ripening a significant increase to a coarser grain type was seen. The rise at 10 wk storage followed by 10 days

ripening of mean sensory response for grain type (Fig. 4) was primarily due to Monroe and Idared cultivars. This phenomenon was in line with actual average particle size measurements as previously noted.

CONCLUSIONS

THE EFFECT of increased ripeness on the quality characteristics of the finished sauce is not necessarily obvious between cultivars or ripening conditions. Flavor quality ratings of the sauce were not affected by any of the treatments in the study. Texture quality ratings were primarily affected by cultivar and ripening treatments and color quality ratings were primarily affected by cultivar and storage treatments. Specific sensory measurements were variously affected by storage and ripening treatments and by cultivar.

These findings indicte that specific postharvest holding conditions can affect individual sensory attributes of the final processed applesauce. Of particular interest is the unusual particle size response of two of the cultivars to long term storage and ripening conditions. Continued work in this area is needed to further understand the trends and why they occur.

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Enzymic Degumming of Pineapple and Pineapple Mill Juices

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————————ABSTRACT————————

Commercial pectinase, cellulase and hemicellulase preparations reduced percent suspended solids, decreased viscosity, and reduced foaming of pineapple juice. These preparations also lowered the viscosity of pineapple mill juice and syrups prepared therefrom to levels approaching sucrose solutions at equivalent Brix. These effects are attributed to enzymic hydrolysis of the neutral polysaccharide pineapple gum and are consistent with the effects of enzymic hydrolyses of purified gum reported previously. Model systems consisting of purified gum in sucrose solution suggest the gum accounts for about 75% and 97% of the nonsugar viscosity of juice, and mill juice, respectively. Ease of filtration, higher clarity, and improved flow are advantages which can be expected with enzymic degumming.

INTRODUCTION

PULP SUSPENSION, viscosity, foaming, and filtration properties of pineapple juice have been attributed to the presence of a natural gum (Dull, 1958; Bates, 1964; Chenchin and Yamamoto, 1978). While pulp suspension and viscosity may be desirable properties for standard juice products, foaming and resistance to filtration are problematic in fermentation, concentration, and filtration unit operations. If clear of low-viscosity products are desired, all properties of the gum may be undesirable and a suitable method for its removal may be needed.

Hydrolysis of natural fruit gum with hydrolytic enzymes to aid filtration and clarification of fruit juices is an old and widely used technology (Nelson and Tressler, 1980; Ishii and Yokostuka, 1972) but its use on pineapple juice has not been reported previously.

Pineapple gum has been isolated and shown to be a neutral polysaccharide composed predominately of galactomannans. The purified gum can be hydrolyzed by commercial pectinase, cellulases and hemicellulase preparations as seen by the loss of functional properties (Chenchin and Yamamoto, 1978). Although hemicellulase was the most effective of the preparations tested, differences in maximal hydrolysis were not large probably owing to heterogeneity of these commercial preparations. Susceptibility of the purified gum to enzymic hydrolysis suggests the feasibility of degumming pineapple juice by direct enzyme treatment. We report herein the effects of direct enzymic treatment of pineapple juice on the various properties attributed to pineapple gum. Also reported is the enzymic degumming of mill juice, a byproduct of pineapple canning, and syrup derived therefrom.

MATERIALS & METHODS

Sample preparation

Pineapple (*Ananas comosus* (L.) Merr. cv. Smooth Cayenne) juice and mill juice were obtained from commercial runs. Concentrates of these juices were prepared on a laboratory rotary evaporator

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(Buchi Rotovapor) under 135 mm pressure at 50°C. Pineapple juice was filtered through a 37μ nylon cloth (Tobbler, Ernest, Traber, Inc.) to remove suspended pulp for viscosity measurements.

Enzyme treatments

Commercial pectinase (Kleerzyme HT), cellulase (Cellzyme 225), and hemicellulase (Rhozyme HP-150) were used. The former two were obtained from Wallerstein Co. and the latter from Rohm and Haas Co. Enzyme concentrations used in the treatments were 300 ppm for Kleerzyme HT and Cellzyme 225 and 50 ppm for Rhozyme HP-150. These concentrations were found in preliminary tests to give the standard treatment condition of maximal viscosity decrease in 10 min at 50°C.

Assays

Relative viscosity, foaming (overrun) and foam stability (drainage) properties were determined as described previously (Chenchin and Yamamoto, 1978). Suspended solids were determined as the percentage volume of solids in 50 mL supernatent from 300 mL pineapple juice which had been left to stand 2 hr in a graduated cylinder (32 x 3.5 cm) at 22°C. Total solids were determined according to the USDA Standards (1956) as percentage volume on 50 mL of thoroughly mixed juice. Filtration rate was determined by gravity filtration of 50 mL juice at 22°C through Whatman No. 1 filter paper (7.5 cm) supported on a Buchner funnel.

RESULTS & DISCUSSION

Effect of enzyme treatment on pineapple juice

Pineapple juice can be modified by enzymic treatment as predicted by previous studies with the purified gum (Chenchin and Yamamoto, 1978). The various enzyme preparations all reduced juice viscosity (Fig. 1). The pectinase treatment achieved the lowest viscosity level followed by cellulase and hemicellulase treatments but the differences in final viscosity levels attained by the various treatments were
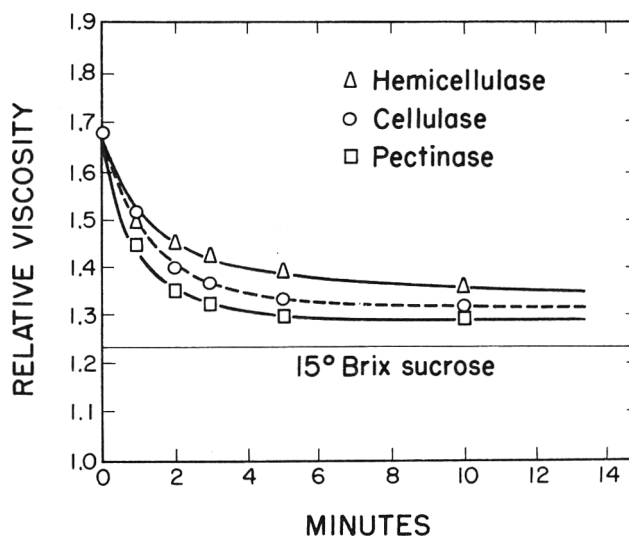
Fig. 1—Effect of hydrolytic enzymes on pineapple juice viscosity. A line representing the viscosity of 15° Brix solution is shown for reference.

not large. In case of the pectinase treatment the initial viscosity was reduced 77%. The fact that the viscosity of 15° Brix sucrose solution was not attained may indicate incomplete hydrolysis of the gum or contributions to viscosity by other factors not affected by enzyme treatment. Incomplete hydrolysis was also observed in enzymic treatment of purified pineapple gum solutions, although the final levels achieved in the model studies were lower (Chenchin and Yamamoto, 1978). Whereas the hemicellulase preparation was the most effective of enzymic preparations examined in the case of purified pineapple gum, it was the least effective preparation in reducing the viscosity of pineapple juice.

The effect of purified pineapple gum concentrations on viscosity of 15° Brix sucrose solution was investigated (Fig. 2). Viscosity increased linearly with gum concentration and at the concentration found in juice (0.054%) attained a viscosity equivalent to 75% of pineapple juice. This is in close agreement with the maximal viscosity reduction observed by enzymic hydrolysis of pineapple juice and shows pineapple gum does in fact account for a large fraction of pineapple juice viscosity. Furthermore, enzymic hydrolysis of gum proceeded to about the same extent in juice as it did in the purified system. The balance of the residual viscosity hence was due to the presence of suspended solids and other nonhydrolyzable polymeric substances.
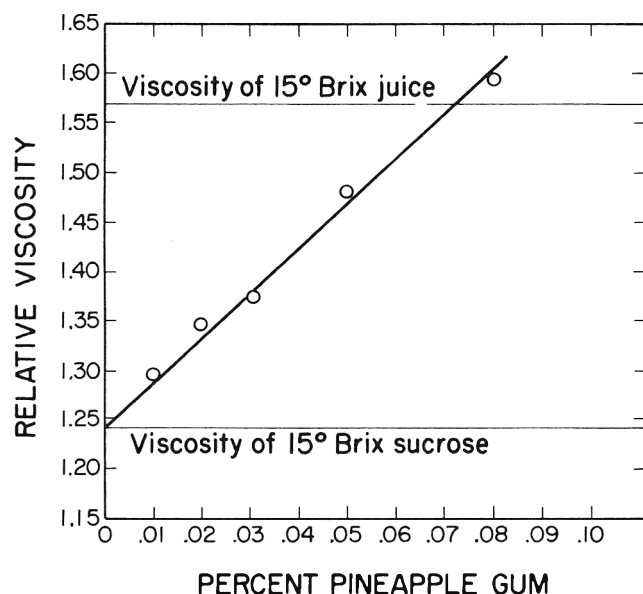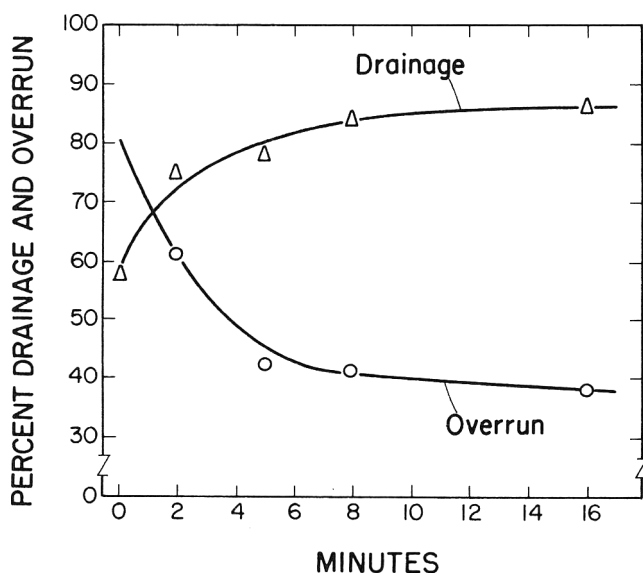
Treatment with pectinase reduced overrun from 80% to 35% with a corresponding reduction of foam stability (Fig. 3). In all three treatments suspended solids decreased approximately 5–10 fold over controls and cloud separation increased (Table 1). In contrast with the untreated juice, filtration rate for the enzyme-treated sample was not only higher but the rate of filtration decreased less rapidly (Fig. 4). Also, the rate of filtration after 15 minutes was approximately 2-fold higher for the enzyme-treated sample than the untreated control. These effects of enzyme treatment appear to be consistent with the effects of enzymic hydrolysis of purified pineapple gum reported previously (Chenchin and Yamamoto, 1978). Enzyme treatments significantly modify the physical properties of pineapple juice and these modifications are due to degumming.

Although the effect of enzyme treatment on the sensory qualities of pineapple juice has not been investigated, effects on mouthfeel may be anticipated. Increased pulp and cloud separation would be undesirable for standard pineapple juice but may be advantageous for production of a clear juice or of pineapple wine. A further application may be the preparation of pineapple concentrates of lower viscosity. Table 2 shows the results of enzymic treatment of two lots of juice and their subsequent concentration, one to 56°

Fig. 2—Relative viscosities of purified pineapple gum solutions in 15° Brix sucrose. The viscosity of 15° Brix pineapple juice is shown for reference.

Table 1—Effect of enzyme treatment on suspended solids of pineapple juice

Juice sample	Suspended solids (%)	Total solids (%)	Relative viscosity[a]	Visual separation[b]
Pectinase-treated	2	22	1.28	+++
Control	19	22	1.73	+
Celulase-treated	3	23	1.35	+++
Control	16	23	2.01	+
Hemicellulase-treated	2.5	20	1.45	+++
Control	15	20	2.14	0

[a] Relative Viscosity at 40°C
[b] 0: No separation between pulp and serum; +: Some separation; +++: Distinct separation

Fig. 3—Effect of pectinase on foaming (overrun) and foam stability (drainage).
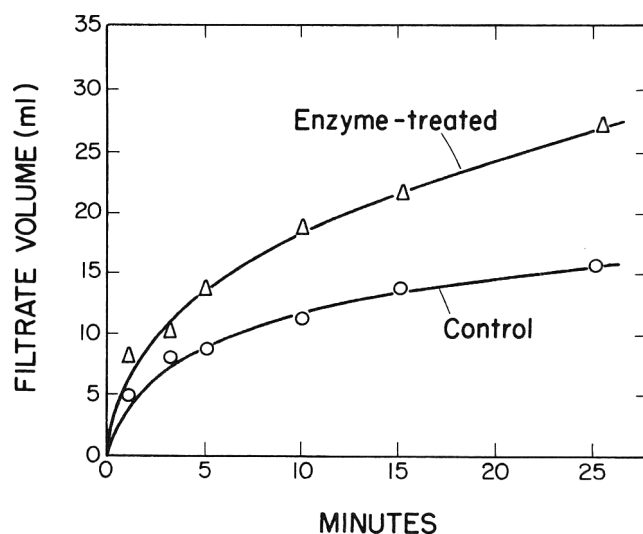
Fig. 4—Effect of pectinase on filtration rate of pineapple juice.

Table 2—Effect of enzyme treatment on viscosity of pineapple juice concentrate

Sample	Juice			Concentrate	
	°Brix	Total solids (%)	Relative viscosity[a]	°Brix	Viscosity[b]
Pectinase-treated	13	16	1.24	56.0	4.7
Control	13	16	1.81	56.0	206.4
Pectinase-treated	13.2	18	1.25	62.6	40.9
Control	13.2	18	1.72	62.6	No flow

[a] Relative viscosity at 40°C
[b] Viscosity as flow in seconds thru a drain tube 150 mm (length) x 8 mm (i.d.) at 22°C

Table 3—Effect of enzyme treatment on viscosity of ion exchange effluents and pineapple syrup

Sample	°Brix	pH	Relative viscosity	
			Before[a]	After[b]
Clarified mill juice	9.5	3.5	1.57	1.15
Cation effluent	9.0	3.5[c]	1.56	1.17
Anion effluent	8.7	3.6[c]	1.57	1.13
Pineapple syrup	14.0	3.6[c]	1.97	1.24
Sucrose solution	9.0	3.4[c]	1.13	1.12
Sucrose solution	14.0	3.5[c]	1.22	1.20

[a] Relative viscosity at 50°C before enzyme treatment
[b] After treatment with 300 ppm Kleerzyme (pectinase) at 50°C for 10 min
[c] pH adjusted with citric acid or 10N NaOH

Table 4—Viscosity of pineapple syrups prepared from enzyme-treated and untreated mill juice

Sample[a]	Relative Viscosity[b]
Mill Juice	
Untreated	1.37
Treated	1.14
20° Brix Syrup	
Untreated	2.89
Treated	1.49
30° Brix Syrup	
Untreated	5.89
Treated	1.95
40° Brix Syrup	
Untreated	14.38
Treated	2.96

[a] Enzyme treatment was with 300 ppm Kleerzyme (pectinase) at 50°C for 10 min
[b] Relative viscosity was determined with a size 200 viscometer at 50°C

Brix and the other to 62.5° Brix. A marked difference in the viscosity of concentrates prepared from enzymically treated juice and untreated control was evident. In the case of 62° Brix concentrate no flow was observed whereas enzymically treated concentrate showed a flow time of about 41 sec.

Effects of enzyme treatment of pineapple mill juice

Pineapple mill juice is extracted from eradicated skins, ends and trimmings and is clarified after heating by filtration through diatomaceous earth. The resulting clarified mill juice is passed through a series of ion exchange resins to remove proteins, organic acids, ash, and color. The clear and neutral effluent, consisting mostly of sugars, is concentrated to produce pineapple syrup. Since pineapple gum is a neutral polysaccharide, these steps would not be expected to remove the gums and accordingly the syrup is highly viscous and often gels when refrigerated.

Enzyme treatment of fractions from various stages in the preparation of syrup or the final syrup lowered the relative viscosity close to sucrose solutions of equivalent Brix (Table 3). The viscosity decrease was equivalent to a 97% reduction in the nonsugar viscosity and comparable to the maximal reduction obtained for enzymic treatment of purified gums (Chenchin and Yamamoto, 1978). Apparently the viscosity of clarified mill juice fractions, except for sugars, is almost entirely due to pineapple gums. Table 4 shows the viscosity of pineapple syrup prepared from pineapple mill juice treated initially with enzyme and then carried through the complete syrup conversion process. As expected the viscosity of pineapple syrup prepared from enzyme-treated mill juice was considerably lower than the untreated control syrup, the difference increasing with syrup concentration.

Enzymic hydrolysis appears to be an effective method for degumming pineapple juice. Degumming has marked effects on viscosity, suspended solids, and cloud stability. These effects may be advantageous for producing clear products and for improving filtration or concentration efficiency. Enzymic degumming may be particularly useful for fermentation processes. This adds to a small but growing body of potential applications for enzymes in tropical fruit processing. Previously Brekke and Meyers (1978) reported guava puree can be treated with pectinolytic enzyme to reduce its viscosity prior to concentration. A 3.9-fold concentrate of enzyme-treated puree was easily pumped and flowed in an evaporator whereas a 3.1-fold concentrate of untreated puree was so viscous that it was difficult to handle

and could be further concentrated. In the case of passion fruit juice, viscosity also limits the degree of concentration which can be attained. The viscosity of passion fruit juice increases rapidly after being heated due to the gelatinization of starch. Kwok et al. (1974) reported that α-amylase was effective in reducing passion fruit juice viscosity after the starches gelatinized.

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Storage Stability of Grapefruit Syrups

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——————————— ABSTRACT ———————————

Ion exchange resins and polymeric adsorbents were used to remove bitterness (flavanones), color destabilizing compounds and microbial growth promoting compounds from clarified grapefruit juice. The syrups prepared from the treated grapefruit sera were stable against color and microbial deterioration in storage.

INTRODUCTION

CITRUS JUICE concentrates at 65° Brix are stable against spoilage from most organisms. Usually only osmophilic yeast tolerate the low water activity (0.78) of high Brix concentrates (Sand, 1973). Susceptibility to nonenzymic browning, however, increases as water activity decreases (Eichner, 1975) so that flavor and appearance of higher Brix concentrates may change considerably. Removal of compounds that promote browning increased color stability of 70° Brix syrup prepared from orange sera treated with cation exchange resin (Bruemmer and Bowers, 1977). Syrups prepared from grapefruit sera can also be stabilized against microbial spoilage and browning in storage. We report and discuss the stability of grapefruit syrups prepared from sera treated to remove amino acids, proteins and the bitter compound, naringin.

MATERIALS & METHODS

FRESHLY EXTRACTED GRAPEFRUIT juice was provided by local processing plants. Polygalacturonic acid was obtained from Kingsley and Keith (Englewood Cliffs, NJ). Irgazyme, a commercial brand of pectinase enzymes, was provided by Ciba Geigy (Greensboro, NC).

Saccharomyces bailii and *S. rouxii* were obtained from the Culture Collection of the USDA, ARS, Northern Regional Research Center (Peoria, IL). Duolite S761 and Duolite A-7(OH⁻) were obtained from Diamond Shamrock (Redwood City, CA), Amberlite XAD-7 from Rohm and Haas, (Philadelphia, PA), and other adsorbents and chemicals were obtained from Sigma Chemical Company (St. Louis, MO).

Preparation of grapefruit serum

Serum was prepared from fresh unpasteurized grapefruit juice by the method used for orange juice serum (Bruemmer and Bowers, 1977). A 3% solution of polygalacturonic acid adjusted to pH 4.0 with 5N NaOH was added to chilled grapefruit juice to final concentration of 300 ppm to aggregate suspended solids. The serum was immediately recovered by centrifugation at 8000 rpm for 2 min in a Lourdes refrigerated Betafuge Model A-2. The yield of serum averaged 86% of the juice.

Preparation of grapefruit syrups

We prepared 10 types of syrups for determining microbial and color stability. The number system is used in Table 1.

To prepare grapefruit syrup 1, we reduced the viscosity of the serum with pectinase (Irgazyme) at final concentration of 300 ppm, and storage overnight at 4°C. Then we pasteurized the treated serum

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at 90°C for 6 sec, cooled it rapidly to 10°C and concentrated it in a Buchi Rotovapor R-20 at 30°C. Syrup Brix values were calculated from weights of serum and syrup and also from values of titratable acidity and sugars. Sugars were measured with an Abbe-3L refractometer (Bausch and Lomb, Rochester, New York). Syrups were stored at 4°C until tested; time of storage was less than 1 month.

Grapefruit syrup 2 was prepared as described for syrup 1 except that after pasteurization and cooling, 6 liters of serum were passed through 1800g Dowex 50W-8x (H⁺ form, 20-50 mesh) in a 5 × 100 cm column at the rate of 20 mL/min. The effluent from this treatment and from other columns described below was monitored for amino acids and proteins by the spot tests [i.e., ninhydrin for amino acids and tetrabromophenolphthalein ethyl ester for protein (Feigl, 1956)] for ascorbic acid (Horowitz, 1970) and for naringin and other flavanones (Davis, 1947) to determine effectiveness of their removal. Flavor and odor changes in the sera after column treatments were monitored by the authors. The holdup serum in the column was washed out with distilled water, combined with the major effluent and concentrated in the Rotovapor at 30°C bath temperature. Brix values were calculated as described for syrup 1.

Grapefruit syrups 3-6 were prepared as described for syrup 2 except that Dowex 1-8 x (OH⁻ form, 20-50 mesh), Duolite A-7 (OH⁻ form), Amberlite XAD-7 and Duolite S761 were used instead of Dowex 50-8x (see Table 1).

Grapefruit syrup 7 was prepared as described for syrup 2 except that activated carbon was used instead of Dowex 50-8x.

Grapefruit syrups 8-12 were prepared as described for syrup 2 except that after the sera were passed through Dowex 50w-8 they were passed through Dowex 1-8x in a column of similar size and conditions. The pH of the syrup was adjusted before storage to 3.0, 4.4, 5.5 and 6.5 from the original 8.9 with N citric acid.

Grapefruit syrup 13 was prepared as described for syrup 2 except that after the serum was passed through Dowex 50W-8 the serum was passed through Duolite S761 in a column of similar size and conditions.

Grapefruit syrup 14 was prepared as described for syrup 13 except that after the serum was passed through Duolite S761 the serum was passed through activated carbon in a column of similar size and conditions.

Table 1—*Naringin content of sera and color stability of syrup after filtration of sera*

Syrup	Treatment	Sera pH	Sera Naringin as flavonones ppm	Syrup color formation[a] ΔC/day at 50°C
1	None	3.2	600	5.12
2	Dowex 50W-8X (H⁺)	2.3	530	1.81
3	Dowex 1-8X (OH⁻)	10.1	0	0.12
4	Duolite A-7 (OH⁻)	7.7	10	1.87
5	Amberlite XAD-7	3.2	10	2.15
6	Duolite S761	3.2	10	2.31
7	Activated carbon	5.0	10	5.00
8	Dowex 50 + Dowex 1	8.9	0	0.01
9	Dowex 50 + Dowex 1	3.0	0	0.01
10	Dowex 50 + Dowex 1	4.4	0	0.03
11	Dowex 50 + Dowex 1	5.5	0	0.13
12	Dowex 50 + Dowex 1	6.5	0	0.17
13	Dowex 50 + Duolite S761	3.0	20	0.51
14	Dowex 50 + Duolite S761 + activated carbon	3.0	0	0.00

[a] Color formation, ΔC/day at 50°C calculated from C = $A_{450} - A_{600}$ × 30 × dilution = color density of a 70° Brix syrup.

Osmophilic yeasts

Inoculated cultures of *Saccharomyces bailii* y-7255, *S. bailii* Y-7262, *S. rouxii* Y-228, and *S. rouxii* Y2547 (NRRC Collection number) were grown to population densities of about 6×10^8 organisms/mL in shake flasks at 100 rpm at 27°C in the following medium (pH 4.5): 0.5% tryptone, 20.1% glucose, and 0.25% yeast extract in distilled water. These cultures were used as inocula (0.1 mL) for 10 ml of the 70° Brix syrups.

Microbial growth test

Syrups inoculated with osmophilic yeasts were incubated for 4, 12 and 20 days at 26°C. They were then diluted 10 and 100-fold with the hyperosmotic growth medium to give colony plate counts in the 30 – 300 range. The growth medium for the yeasts containing 3% agar was used as pour plates for growth test of the yeasts in the syrups. Three plates were poured for each dilution of each syrup. Plates were examined and colonies counted after 48 and 96 hr at 30°C.

Measurement of color formation

The official method for analyzing corn syrup color was modified for use with grapefruit syrups (Haynak and Bollenback, 1966). Syrups were diluted to 5° Brix at 20°C. Absorbance values at 450 nm and 600 nm were determined with a Beckman DU spectrophotometer. When the 450 nm absorbance value was greater than 0.5, the syrup was diluted 1 to 10 by volume, or more if necessary, to bring the absorbance below 0.5 nm. The dilution factor includes 30 (to revert observed color to 40 commercial Baume concentration of original) times the number of dilutions made to read on scale. Solution color = $(A_{450} - A_{600}) \times$ (dilution factor). The solution color was used to determine the rate of color development in the syrups.

RESULTS & DISCUSSION

THE ANION EXCHANGE RESINS, Dowex 1-8x and Duolite A-7, and the adsorbents, Amberlite XAD-7, Duolite S761 and activated carbon removed naringin from grapefruit sera (syrups 3-7, Table 1). Dowex 50W-8x and Dowex 1-8x and Duolite A-7, but not adsorbents, also removed amino acids (data not shown in table). Dowex 1-8x was most effective of the individual ion exchange resins in removing color destabilizing compounds (compare syrup 3 with syrups 2 and 4 for color formation, Table 1). Dowex 1 removed amino acids which are known to react with hex-

oses to form Maillard browning products (Spark, 1969) and also removed ascorbic acid and other organic acids which are reactants in browning reactions (Shaw et al., 1977). Duolite A-7 also removed anions but not as effectively as Dowex 1 as evidenced by smaller change in pH after Duolite A-7 treatment. Duolite S761, a protein absorbent, lowered the rate of color development about 50% (syrup 6, Table 1) but was most effective when the sera was first filtered through Dowex 50 (syrup 13) and also through activated carbon (syrup 14). The two-step treatment of filtration through Dowex 50 and then Dowex 1 was very effective in stabilizing syrup color (syrups 8 through 12) because the treatment removed amino acids and ascorbic acid reactants in the browning reactions. The pH of the syrup had a slight effect on stability; syrups at pH extremes (pH 3, 4.4 and 8.9) were more stable than the slightly acid syrups (pH 5.5 and 6.5).

Treatment of sera with Dowex 50W-8x lowered the pH and treatment with Dowex 1-8x, Duolite A-7 and activated carbon elevated the pH. Amberlite XAD-7 and Duolite S761 had no effect on pH. The sugar content of the sera was not affected by filtration through the columns. No off-flavor resulted from the treatment.

Activated carbon effectively removed naringin from sera after batch treatment for 30 min (Table 2). Naringin was reduced more than 50% with 0.5g activated carbon per 100 mL serum. At 2.0g per 100 mL the naringin was reduced to less than 100 ppm. No adverse odor or flavor was imparted to the serum by activated carbon treatment.

Syrups at 70° Brix did not support growth of *Saccharomyces* at 26°C (Table 3). Four days after inoculation with 6×10^6 organisms/mL, syrups prepared from sera filtered through both Dowex 50 and Duolite S761 and through three columns, Dowex 50, Duolite S761 and activated carbon, contained less than 10 viable organisms per mL. Syrups prepared from sera filtered through Dowex 1 and through both Dowex 50 and Dowex 1 maintained viable organisms for the 4 and 12-day test period but few organisms were detected after 20 days storage. Seventy °Brix syrup prepared from unfiltered serum supported growth of all cultures and produced gas by 20 days (data not shown in table). Thus, filtering the sera through exchange resins and adsorbents removed growth-promoting substances and stabilized the syrup against microbial degradation.

CONCLUSION

THE ANION EXCHANGE RESINS, Dowex 1-8x and Duolite A-7 and the adsorbents Amberlite XAD-7, Duolite S761 and activated carbon effectively removed naringin and other flavanones from grapefruit sera. Dowex 1-8x alone and when following filtration through Dowex 50 effectively removed the color destabilizing compounds from grapefruit sera. The ion exchange resins and the adsorbents effectively removed growth-promoting substances for the *Saccharomyces* from grapefruit sera. Treatment of grapefruit sera with a combination of ion exchange resins and adsorbents protects syrups prepared from the sera against loss of quality from chemical and microbial degradation. These syrups might be useful as sweetening base for fruit beverage formulation.

Table 2—Batchwise removal of naringin from grapefruit sera with activated carbon

Activated carbon	Naringin (ppm) after treatment		
g/100 mL	30 min	60 min	90 min
None	608	580	560
0.5	297	267	243
1.0	167	158	151
2.0	84	72	44

Table 3—Changes in population of Saccharomyces in 70° Brix syrups at pH 7.0 and 26° C

Sera treatment[a]	Incubation (days)	Microbial population (organisms/mL)			
		Y7255	Y7262	Y228	Y2547
Dowex 50 then Dowex 1	4	2×10^4	2×10^4	3×10^4	4×10^5
	12	2×10^4	2×10^2	1×10^4	3×10^3
	20	20	10	40	40
Dowex 1	4	2×10^4	0	2×10^4	1×10^4
	12	4×10^3	0	4×10^3	4×10^2
	20	0	0	0	0

[a] Inoculated to contain 6×10^6 organisms/mL: plate counts in syrups (pH 3.0) prepared from sera filtered through both Dowex 50 and Duolite S761, and through three columns, Dowex 50, Duolite S761 and activated carbon, were less than 10 cells/mL after 4 days.

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Determination of Lactose and Sucrose Contents of Ice Cream Mix via Enzymatic-Cryoscopic Methodology

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─────── ABSTRACT ───────

A simple method was developed for determining the lactose and sucrose contents of ice cream mix. The method is based on measuring the freezing point depression resulting from incubating samples with invertase (EC 3.2.1.26) and β-galactosidase (EC 3.2.1.23). Sucrose and lactose concentrations are determined separately with each determination requiring 50 units of enzyme and 2 hr of incubation at 37°C. A standard curve for lactose determination was obtained using ice cream mixes containing various amounts of sucrose and corn syrup solids ($R^2 = 0.996$, C.V. = 1.2%). A standard curve for sucrose determination was obtained using ice cream mixes containing various amounts of lactose and corn syrup solids ($R^2 = 0.996$, C.V. = 1.9%).

INTRODUCTION

ICE CREAM usually contains a mixture of sugars including lactose, sucrose, and corn syrup solids. To produce a product with consistent flavor and texture, it is useful to determine the concentration of these sugars in the finished product. Methods currently used for analysis of sugar mixtures include paper chromatography (Chou and Tobias, 1959), high performance liquid chromatography (Euber and Brunner, 1979), gas-liquid chromatography (Adachi and Yamaji, 1978), and enzymatic methods utilizing β-galactosidase (EC 3.2.1.23) for lactose hydrolysis, and either glucose oxidase (EC 1.1.3.4), glucose peroxidase (EC 1.11.1.7) or hexokinase (EC 2.7.1.1) for glucose determination (Frater et al., 1977, Cheng and Christian, 1977). These methods are not suitable for routine use in many quality control laboratories because of the expense, expertise, or time required. The lactose content of ice cream cannot be measured by chemical procedures such as those developed by Teles et al. (1978) and Nickerson et al. (1976) because of interference from maltose and glucose. The titrimetric method developed by Peeples and Hutcheson (1978) for determining sucrose and lactose in ice cream is somewhat time consuming for routine use.

Since hydrolysis of a disaccharide to its monosaccharide components results in a depression of the freezing point of the solution in direct proportion to the moles of monosaccharide released, a cryoscope can be used to monitor the enzymatic hydrolysis of lactose (Ramet et al., 1979, Baer et et al., 1980, Chen et al., 1981) and to determine the lactose concentration of dairy products (Zarb and Hourigan, 1979). The same principle can be applied to determine sucrose concentration.

This paper describes a simple, economical method for determining lactose and sucrose concentrations in ice cream based on measuring the freezing point depression resulting from enzymatic hydrolysis of the two disaccharides.

MATERIALS & METHODS

Enzymes

Escherichia coli β-galactosidase (β-D-galactoside galactohydrolase, EC 3.2.1.23) was obtained from P-L Biochemicals as a par-

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tially purified powder with an activity of 50 units/mg. Grade VII (partially purified powder) Bakers yeast invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) with an activity of 400 units/mg was obtained from Sigma Chemical Co. Both enzymes were diluted to 1 unit/μL using 0.1M potassium phosphate buffer (pH 7.3).

Lactose procedure

Ice cream was diluted by weighing 4.17g into a 25 mL volumetric flask and bringing it up to volume with distilled water. Three mL of this dilution were pipetted into a clean, dry cryoscope tube and 50 μL β-galactosidase solution (1 unit/μL) added. The freezing point was determined immediately after mixing and the tube transferred to a 37°C water bath for hydrolysis. After 2 hr of incubation, the second freezing point was determined. The freezing point depression (ΔT) resulting from lactose hydrolysis was calculated in degrees Hortvet (°H). Each analysis was done in duplicate.

Sucrose procedure

Ice cream was diluted by weighing 5.00g into a 25 mL volumetric flask and bringing it up to volume with distilled water. Diluted ice cream (1.5 mL) was added to the cryoscope tube and mixed with 1.5 mL 0.05M potassium acetate buffer (pH 4.8). Fifty μL invertase solution (1 unit/μL) were added. The freezing point was measured immediately after mixing. The reaction mixture (pH 5.1) was then placed in a 37°C water bath for 2 hr. The second freezing point was measured and the freezing point depression (°H) resulting from sucrose hydrolysis was calculated. Each analysis was done in duplicate.

Ice cream mix

The basic ice cream mix used in developing and testing the standard curves was made from fresh whole milk, heavy cream, nonfat dry milk, and stabilizer (NPL56, Dari-Tech, Atlanta, GA). The stabilizer contained cellulose gum, mono- and diglycerides, guar gum, polysorbate 80, and carrageenan. The mix was formulated to contain 10% fat, 19.8% total solids, and 5% lactose. Fat content was determined by the Babcock method (Atherton and Newlander, 1977), total solids by the Mojonnier method (Atherton and Newlander, 1977), and lactose content by the method of Nickerson et al. (1976). The mix was pasteurized, homogenized and stored frozen until used.

Determination of standard curves

Various amounts of sugars were added to the basic ice cream mix at the time of dilution for sugar analysis. Sucrose was added to achieve concentrations of 6-18% in the undiluted mix. Thirteen different sucrose concentrations in 1% increments were analyzed within this range. Samples containing each sucrose concentration were analyzed in the presence of 5, 7 and 9% lactose, and 4 and 7% corn syrup solids (DE = 36). The lactose standard curve was developed in a similar manner, by adding lactose to the mix to achieve concentrations of 5.0-9.0% in 0.5% increments. Samples containing the various lactose concentrations were analyzed in the presence of 11, 15 and 18% sucrose and 4 and 7% corn syrup solids. The freezing point depressions resulting from sugar hydrolysis were calculated for each sample. Data were analyzed for the effect of the various sugars on replication by Analysis of Variance ($\alpha = 0.05$). Means were separated using Duncan's multiple range test ($\alpha = 0.05$). Regression equations were derived for the calculation of lactose and sucrose concentrations from freezing point depression.

The effect of fructose on sucrose analysis was determined by analyzing ice cream mixes containing 6, 8, 10, and 12% sucrose with and without 4% added fructose.

Verification of regression equations

The regression equations were tested for predictive ability by presenting the analyst with 18 unknown samples in random order. These samples contained various amounts of sucrose, lactose, and corn syrup solids, and were prepared similarly to those used in developing the standard curve. All analyses were done in duplicate. The standard error of prediction for both lactose and sucrose analysis was calculated using these data.

Analysis of commercial ice cream

Samples of eight brands of vanilla ice cream were purchased at three different times over a period of several months. Thus, the compositional variation within each brand could be estimated. The sucrose and lactose contents of these samples were determined by using the described method. Fat and total solids content were determined as described for the laboratory prepared mix.

RESULTS & DISCUSSION

Sucrose analysis

The standard curve developed for determining sucrose is given in Fig. 1. This figure also illustrates the range of freezing point depressions resulting from the analysis of ice cream mixes containing different amounts of lactose (5, 7, and 9%) and corn syrup solids (4 and 7%). An Analysis of Variance of these data indicated a significant effect of mix formulation; the mixes containing 4% corn syrup solids exhibited the greatest mean freezing point depression (0.067 °H) and the mixes containing 9% lactose exhibited the smallest mean freezing point depression (0.0592 °H). Since the difference between these two means is only 0.0015 °H, and since the cryoscope can detect a difference of only 0.001 °H, it is our opinion that interference from

corn syrup solids and lactose is not of practical significance in this procedure. The statistical significance of such small differences in mean values emphasizes the high degree of precision associated with using the crysocope for measuring disaccharide hydrolysis. Consequently, all data were combined into one standard curve as shown (Fig. 1). Analysis of the combined data showed all sucrose levels to be significantly different, indicating that the method readily detects 1% differences in sucrose concentration.

The equation of the regression line calculated from the sucrose standard curve data was % Sucrose = 1.22 + 180 (ΔT). The standard error of estimate of the intercept was 0.085 and the standard error of estimate of the slope was 1.35. R^2 was 0.996 and the coefficient of variation was 1.9%.

Data on prediction of the sucrose concentration of unknown ice cream samples using the regression equation are presented in Table 1. The mean predicted sucrose concentration was 0.09% lower than the mean true value. The standard deviation of the difference was 0.09%.

Since fructose is an end product of sucrose hydrolysis, there is a possibility that fructose would effect invertase activity. Data in Table 2 show that the depression of freezing point resulting from hydroysis of sucrose was not significantly affected by the addition of 4% fructose to the ice cream mix. The difference in freezing point depression between samples with and without fructose was less than the Least Significant Difference value of 0.0012 °H (α = 0.05) for each concentration of sucrose tested. This result indicates that the presence of high fructose corn syrup will not interfere with the analysis.

Lactose analysis

The standard curve developed for determining lactose is given in Fig. 2. The graph also illustrates the range of the

SUCROSE STANDARD CURVE

%Sucrose=1.22 + 180 (ΔT)

R^2 =0.996

ΔT (°H)
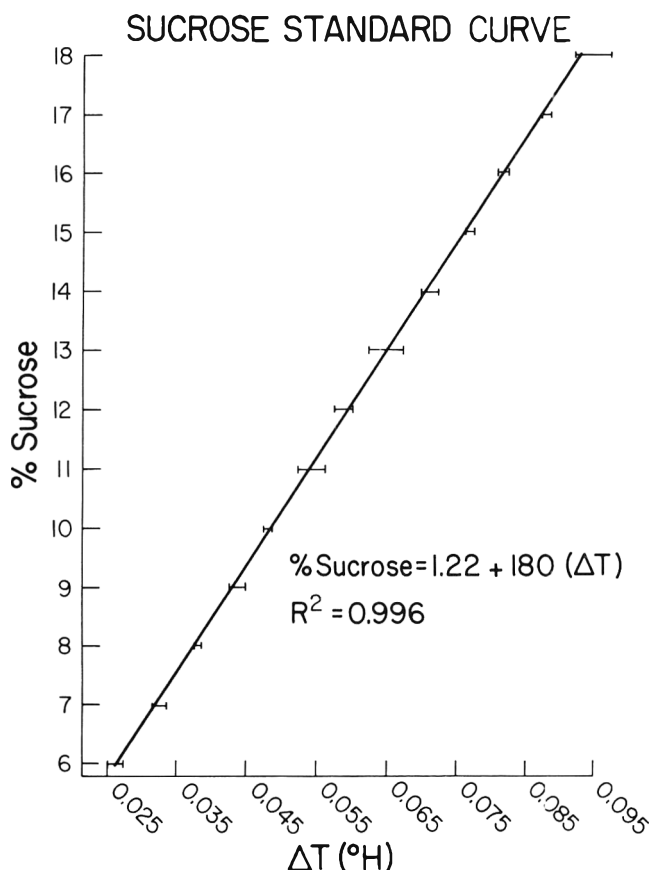
Fig. 1—Standard curve for the determination of sucrose in ice cream using the enzymatic-cryoscopic method. The five replications included various lactose and corn syrup solids levels.

Table 1—Prediction of sucrose concentration using regression equation

	True sucrose	Predicted sucrose	Difference
	(%)	(%)	(%)
	6.7	6.6	−0.1
	7.7	7.7	0
	9.0	8.9	−0.1
	10.1	10.2	+0.1
	11.1	11.0	−0.1
	12.5	12.4	−0.1
	14.5	14.3	−0.2
	16.4	16.2	−0.2
	17.5	17.4	−0.1
Mean	11.72	11.63	−0.09

Table 2—Effect of addition of fructose on freezing point depression resulting from sucrose hydrolysis

	Mean[a] freezing point depression (°H)[b]	
	Fructose (%)	
Sucrose (%)	0	4
6	0.027	0.027
8	0.038	0.037
10	0.047	0.048
12	0.058	0.057

[a] n = 2
[b] °H = degrees Hortvet

LACTOSE STANDARD CURVE

$$\% \text{Lactose} = -.144 + 107\,(\Delta T)$$
$$R^2 = 0.996$$

Fig. 2—Standard curve for the determination of lactose in ice cream using the enzymatic-cryoscopic method. The five replications included various sucrose and corn syrup solids levels.

freezing point depressions resulting from the analysis of ice cream mixes containing different amounts of sucrose (11, 15, and 18%) and corn syrup solids (4 and 7%). In contrast to the sucrose data, the Analysis of Variance of these data revealed that the various amounts of sucrose and corn syrup solids had no significant effect on lactose determination. Each level of lactose was significantly different from the others — demonstrating that the method readily detected 0.5% differences in lactose concentration. The regression equation calculated for lactose determination was % Lactose = $-0.144 + 107(\Delta T)$. The standard error of the estimate of the slope was 1.04. R^2 was 0.996 and the coefficient of variation was 1.2%.

Table 3 presents data on the use of the lactose regression equation for the prediction of unknown samples. Mean predicted lactose concentration was 0.022% higher than the true values. The standard deviation of the difference was 0.09%.

Application to commercial ice cream

Results of a compositional analysis of eight different brands of vanilla ice cream are presented in Table 4. Three different sampling times were used for each brand, so that variation between batches could be estimated. Samples of known sugar concentration were analyzed with each set of commercial samples to verify the continued accuracy of the procedure. These control samples all conformed to the previously stated limits of the method. Mean lactose concentrations of the commercial samples ranged from 5.4-6.3% and mean sucrose concentrations from 6.3-12.3%. Three brands (C, G, and H) had standard deviations of sucrose content close to 1%. It is not known whether this lack of uniformity was intended or due to poor formulation controls. Causes for unwanted variations in sugar content include variations in liquid sugar composition and imprecise metering devices. Brand G also showed substantial variation in fat content (10.0% ± 0.87). Brands A and F had very uniform products with regard to sucrose, lactose, and fat content. All ten brands tested met the minimum legal requirement for fat content of 10.0%, although Brand G was marginal in this respect. The mean solids-not-fat of the commercial samples ranged from 26.3-29.3% and the mean total solids ranged from 37.3-39.6%.

The cryoscopic method for determining lactose and sucrose in ice cream should be of use to those manufacturers interested in marketing a product of consistent composition. The method is both accurate and precise enough for this purpose. Most dairy quality control laboratories already have the capability to determine freezing points. The only other equipment needed is a water bath and standard laboratory glassware. The major nonlabor cost is that of the lactase at $0.46/duplicate analysis. Less expensive lactase enzyme preparations designed for dairy industry use cannot be used for this method since they may contain substantial invertase activity.

Table 3—Prediction of lactose concentration using regression equation

	True lactose	Predicted lactose	Difference
	(%)	(%)	(%)
	5.2	5.2	0
	5.9	5.8	−0.1
	6.0	6.0	0
	6.3	6.3	0
	7.0	7.0	0
	7.7	7.9	+0.2
	8.0	8.1	+0.1
	8.5	8.6	+0.1
	8.7	8.6	−0.1
Mean	7.03	7.06	+0.03

Table 4—Composition of eight commercial ice cream samples.

Brand	Lactose	Sucrose	Fat	Solids-not-fat	Total solids
	(%)	(%)	(%)	(%)	(%)
A	6.3 ± 0.10[a]	8.1 ± 0.15	10.3 ± 0.29	27.1 ± 0.70	37.4 ± 0.99
B	6.0 ± 0.72	11.3 ± 0.64	10.3 ± 0.44	27.3 ± 0.55	37.6 ± 0.21
C	5.8 ± 0.44	6.3 ± 1.1	10.3 ± 0.10	29.3 ± 0.67	39.6 ± 0.78
D	5.6 ± 0.21	8.0 ± 0.66	10.6 ± 0.15	27.7 ± 1.4	38.2 ± 1.2
E	5.5 ± 0.29	12.3 ± 0.52	11.0 ± 0.06	26.3 ± 1.1	37.3 ± 0.96
F	5.4 ± 0.26	12.1 ± 0.23	11.4 ± 0.06	26.7 ± 1.4	38.2 ± 1.3
G	6.0 ± 0.26	8.4 ± 1.2	10.0 ± 0.87	27.9 ± 0.95	37.9 ± 0.50
H	5.4 ± 0.15	12.3 ± 0.90	11.1 ± 0.06	26.9 ± 0.17	38.0 ± 0.15

[a] n = 3; variation calculated from difference between samples purchased on three different occasions.

Nonenzymatic Browning in Pear Juice Concentrate at Elevated Temperatures

T. BEVERIDGE and J.E. HARRISON

———————————— ABSTRACT ————————————

The effect of temperature and soluble solids (°Brix) on nonenzymatic browning in pear juice concentrate was determined by following absorbance at 420 nm (A_{420}) over the temperature range of 50-80°C. Browning could be modeled as a zero order rate process with rates of 22.2×10^{-4} (45.2 °Brix), 36.9×10^{-4} (55.4 °Brix), 53.5×10^{-4} (65.1 °Brix) and 107×10^{-4} (72.5 °Brix) $A_{420} \cdot min^{-1}$ at 80°C. Temperature dependence was described by the Arrhenius relationship with an average activation energy of 21.9 kcal $\cdot$ mole^{-1}. Formol titration indicated a 20% loss of amino acids during heating 4.4 hr at 80°C and no loss of carbohydrates was observed after any heating period.

INTRODUCTION

AS A MEANS OF UTILIZING cull pears, production of pear juice concentrate for use as a syrup base for fruit canning, offers an attractive alternative to use as animal feed. Pear juice concentrate is commonly produced as an amber-brown syrup which has a strong tendency to darken during storage thus reducing its usefulness, versatility and economic value. The amber-brown color developed during processing has been ascribed to polyphenol oxidase activity (Luh, 1980) but storage developed color results from non-enzymatic browning reactions (Cornwell and Wrolstad, 1981).

Nonenzymatic browning is a carbohydrate dehydration reaction (Greenwood and Munro, 1979), accelerated through the intervention of amino compounds. Reaction rates depend on several factors including temperature, reactant concentration, pH, water activity and specific ion concentrations (Adrian, 1982), and results in progressive development of brown premelanoidin pigments in the affected food system. Brown color development, as measured as absorbance at 420 nm (A_{420}) frequently can be described by models assuming zero order reaction kinetics (Stamp and Labuza, 1983; Labuza, 1972) perhaps following an initial lag period (Saguy et al., 1978) during which sugar-amine addition products and other color precursors are formed. Kinetic descriptions can be used to predict brown color development during thermal processes and/or storage.

In spite of its importance to the fruit industry, browning of pear juice concentrate has received very little attention. Virtually, none of the available literature deals quantitatively with the rate of color development. The purpose of the present paper is to describe quantitatively, brown color development in pear juice concentrate with particular reference to processing temperatures, and with a view to prediction of storage life.

MATERIALS & METHODS

PEAR JUICE CONCENTRATE was obtained from Sun-Rype Products Ltd. (Kelowna, B.C) as a carbon decolorized, 65.1° Brix syrup

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produced from d'Anjou pears. Other concentrates were prepared by concentrating this syrup on a rotatory evaporator at 40°C or by dilution with distilled water. The pH of the syrups was 4.2. Analysis of variance and Duncan's New Multiple Range tests were performed according to Li (1964).

Browning rate measurement

Concentrate, at room temperature, was divided into 30 mL aliquots contained in 40 mL screw-capped plastic centrifuge tubes. In a separate experiment, it was determined that 20-30 min was required to heat 30 mL of concentrate to bath temperature. Consequently, the rack of prepared tubes was placed in a thermostated water bath and held 30 min, at which time the initial (0-time) samples were withdrawn, cooled in running tap water (10°C) and their A_{420} determined with a Unicam SP1800 spectrophotometer in 1 cm cells.

Water activity

Measured with a Rotronic Hygroskop Water Activity Meter by methods described by the manufacturer.

Amino acids

Measured by formol titration methods modified from AOAC (1975). Titration was performed with 0.5N NaOH using a model TTT80 titrator fitted with a model PHM 82 pH meter (Radiometer, Copenhagen). The procedure was standardized against glycine and results reported in terms of glycine.

Carbohydrates

Measured using a Water's Associates liquid chromatograph equipped with a Water's carbohydrate column and refractive index detector. Sugars were separated isocratically in 85% (v/v) aqueous acetonitrile flowing at 1.5 mL/min utilizing ribose as an internal standard.

RESULTS & DISCUSSION

BROWN COLOR DEVELOPMNET during thermal treatment at 80°C, as measured by A_{420}, was linear with time (Fig. 1) over all solids levels tested. At 70, 60 and 50°C, similar linear relationships were also obtained over testing periods of 7.5 hr, 31.8 and 31 hr. respectively. The linear relationships could be well described by assuming zero order kinetics (Fig. 1, Table 1) in agreement with previous reports on nonenzymatic browning in other systems (Labuza, 1972; Stamp and Labuza, 1983).

No initial lag period such as that reported in sugar-glycine systems (Reyes et al., 1982) was observed. Since the concentrate was initially brown, it is probable that the potentiation reactions that occur during this lag phase, were initiated during juice pasteurization and evaporation to concentration. In this event, no lag period would be expected. Also, the samples were brought to bath temperature over a 30 min holding period. A lag period could be masked by this heating period.

Temperature dependence of the reaction rate constants was well described by the Arrhenius relationship (Fig. 2). The activation energy (E_a) averaged 21.9 k·cal·mole^{-1}, a value somewhat low when compared to those obtained for solid foods of relatively low moisture content (Labuza, 1972), but in general agreement with more liquid systems. Thus the temperature dependence of browning is less in
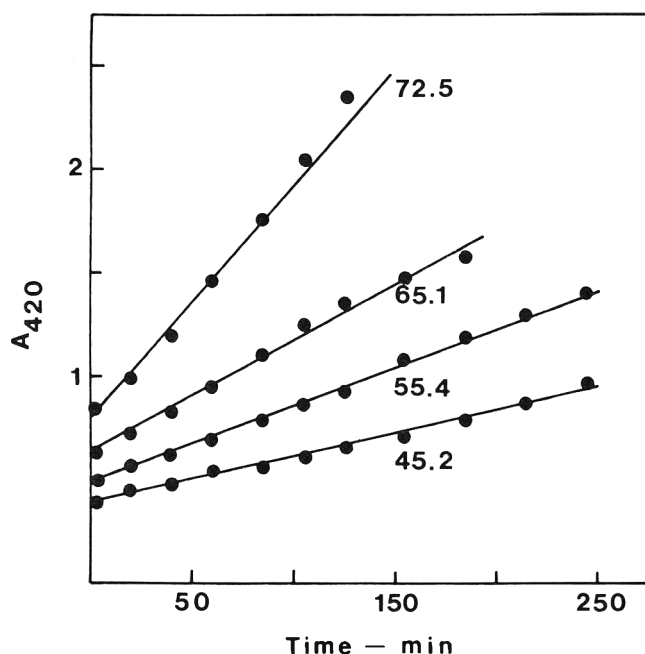
Fig. 1—Browning of pear juice concentrate at 45.2, 55.4, 65.1 and 72.5 °Brix as measured by increased absorbance at 420 nm. Temperature was 80°C.

Table 1—Water activities, zero order rate constants (k x 10^4) A$_{420}$·min^{-1} and Arrhenius activation energies (E$_a$) for browning in pear juice concentrate

Temp (°C)	° Brix			
	45.2	55.4	65.1	72.5
80	22.2 (0.99)[a]	36.9 (0.99)	53.5 (0.99)	107.0 (0.98)
70	7.53 (0.99)	13.9 (0.99)	24.4 (0.99)	43.2 (0.99)
60	4.22 (0.99)	6.43 (0.99)	11.1 (0.99)	15.2 (0.99)
50	1.16 (0.99)	1.72 (0.99)	2.62 (0.99)	4.60 (0.98)
E$_a$ (k.cal. mole^{-1})	20.8	22.0	21.8	23.2
Water activity	0.899	0.851	0.767	0.666

[a] Number in brackets is the correlation coefficient.

these fluid intermediate moisture systems than would have been predicted from previous studies of solid systems.

Plots of zero order rate constants as a function of water activity (Table 1, plots not shown) showed that reaction rates increased as water activity decreased, as expected (Labuza, 1972), however, the anticipated exponential relationship (Eichner and Karel, 1972) was only suggested visually at 80°C. At all temperatures used, over the range of a$_w$ tested, reaction rates could be related to a$_w$ by simple linear regression (R^2 = 0.977-0.998), including the relationship at 80°C. However, the slope of the linear relationship obtained at 80°C was 24.2 times that obtained at 50°C showing that browning rates increase much more rapidly with decreasing a$_w$ at high temperatures than at low. This could have important implications for storage studies at lower temperatures. Perhaps, under low temperature conditions, a$_w$ is not a particularly important parameter; however, at 50°C the rate increase, when solids are increased from 45.2° Brix to 72.5° Brix, is still almost four-fold.

Carbohydrate analysis performed prior to and at the end of each heating period gave pooled average values of 541 ± 35 mg/100 mL/°Brix glucose, 673 ± 37 mg/100 mL/°Brix
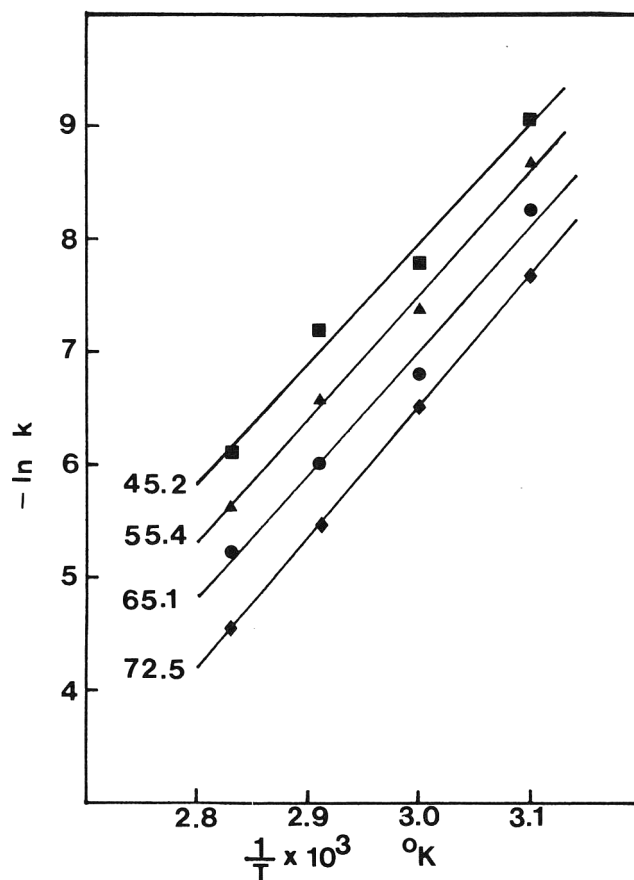
Fig. 2—Arrhenius plot for browning of pear juice concentrate at 45.2, 55.4, 65.1, and 72.5 °Brix over the temperature range 50-80°C. Correlation coefficient for all lines is 0.99.

fructose and 19 ± 9 mg/100 mL/°Brix sucrose. Ranges shown are standard deviations. The low sucrose value indicates inversion under the acidic conditions. Since the methodology used here is unable to separate glucose and sorbitol, the value of glucose given here should be interpreted as glucose and sorbitol measured as glucose. In addition, a trace component which co-eluted with xylose was observed. No loss of either glucose or fructose (P ⩾ 0.05) was detected between initial and final values at any holding temperature or solids content tested in the present study; therefore, pooled values are reported.

Formol titration for amino acids suggested that some consumption of amino compounds occured during browning (Table 2). However, a significant decrease was only observed after heating at 80°C where a decrease of about 20% was noted at the higher solids levels. This consumption was expected since amino compounds participate in the initial carbohydrate dehydration reactions initiating browning. In pears, the levels of these amino compounds may prove very important to storage browning of concentrates since they may occur in both large and variable quantities (Cornwell and Wrolstad, 1981; Burroughs, 1970; Sumbali and Mehrotra, 1982).

Very little literature data are available concerning the rate of brown color development in pear juice concentrate; however, an estimate of 9.3 x 10^{-6} A$_{420}$ · min^{-1} can be obtained from the data of Akhavan and Wrolstad (1980) and one of 4.8 x 10^{-5} A$_{420}$ · min^{-1} from Cornwell and Wrolstad (1981). Both estimates are for a 72° Brix concentrate stored long term (15 - 28 wk) at 37°C, but the former value was obtained with a concentrate prepared from an equal mixture of Bosc and Comice pear juice concentrate. A value near 1.1 x 10^{-4} A$_{420}$ · min^{-1} may be

Color Stability of Astaxanthin Pigmented Rainbow Trout Under Various Packaging Conditions

HUEI-MEI CHEN, S. P. MEYERS, R. W. HARDY and S. L. BIEDE

ABSTRACT

Astaxanthin-rich oil extract from Louisiana crawfish by-product imparted a salmon-red coloration to the flesh and integument of rainbow trout on a diet containing 45 mg astaxanthin/kg feed. Carotenoids deposited in fish flesh were stable (ca 90% retention) in air-packed samples at 1 - 2°C. Oxygen-evacuated and CO_2-enriched packaging gave adverse color stability. Lowest pigment levels were in frozen samples, with 53% of the absorbed pigment degraded in 14 days. Visual color scores were consistent with quantitative pigment analyses ($r = 0.77$). Higher pigment contents were observed in air-packed (1 - 2°C) samples, possibly due to astacene or other breakdown products of astaxanthin. Maximal TBA values were observed in air-packed (1 - 2°C) samples. The effect of tissue enzyme homogenate on fading of trout flesh is discussed.

INTRODUCTION

THERE IS increasing evidence that the presence of carotenoid pigments directly influences red coloration of salmonid fish flesh/integument and ultimate consumer acceptability of the product (Simpson, 1982; Sceurman et al., 1979; Ostrander et al., 1976). Since freshwater and marine fish, like other animals, cannot synthesize carotenoids de novo, these tissue pigments are derived from dietary sources (Tanaka, 1978; Brinchmann, 1967). Considerable work has demonstrated the feasibility of incorporation of synthetic canthaxanthin or natural astaxanthin in a meal or oil extract form into various dietary formulations for effective coloration of salmonids and crustaceans (D'Abramo et al., 1983; Spinelli and Mahnken, 1978; Bauernfeind, 1976). In addition to the demonstrated influence of astaxanthin on visual appearance, other potentially significant biological and metabolic roles also have been ascribed to this carotenoid during fish cultivation and embryonic development (Meyers and Chen, 1983).

Stability of the deposited carotenoids during transportation, storage, and cooking is critical to assure optimal acceptance of aquaculturally raised products. It has been noted that induced pigmentation of rainbow trout flesh by supplemental dietary canthaxanthin was more stable to heat processing than the natural pigment present in coho salmon flesh (Schmidt and Baker, 1969). In contrast, Spinelli (1978) demonstrated that during cooking, consistently more color fading occurred in rainbow trout fed a diet containing canthaxanthin than those on a zooplankton-supplemented diet. However, quantitative information on storage stability of red coloration in pen-reared salmonids has not been reported to date.

The purpose of this study was to investigate various packaging methods, i.e., air-impermeable, oxygen-evacuated, and CO_2-enriched packs, for preservation of the red color of astaxanthin-pigmented rainbow trout during re-frigerated and frozen storage. Sensory evaluation of raw and cooked fillets, combined with pigment content analyses, were used to assess the pigmentation values of the fish.

MATERIALS & METHODS

Feeding experiment

Twenty-five rainbow trout (Salmo gairdneri), averaging 250g each, were placed into a 700L, fiberglass indoor tank in the fish rearing laboratory of the National Marine Fisheries Service (Seattle, WA). The tank was supplied with 6 L/min constant temperature (15°C), dechlorinated city water. A natural photoperiod was followed. The fish were fed a dry pellet comprising a modified formulation of the Abernathy diet S8-1. The formula was modified by replacing 6% of the 9% added oil with astaxanthin-enriched soy oil extract (750 ppm astaxanthin) prepared from crawfish waste (Procambarus clarkii) by the procedure of Chen and Meyers (1982). This resulted in a supplemental astaxanthin level of 45 mg/kg feed. The diet was pelleted into appropriate sizes using a laboratory-scale California pellet mill and stored at room temperature until used. The feed was made monthly to avoid prolonged storage. The fish were fed at a rate of 1.5% body weight per day in two feedings, 5 days per week for 4 months. At the end of the feeding period, 12 fish with red coloration on the lateral line and fins were removed from the tank, sacrificed, and gutted. Three were stored frozen and the remainder were maintained on ice for 3 days and 1 day, respectively, before packaging treatments.

Packaging and storage condition

All trout samples were washed thoroughly and heads and tails removed. Nine fresh trout were divided into three treatment groups, i.e., air, oxygen-evacuated, and CO_2-enriched packaging. Within each group, individual fish were cut into four sections and randomly assigned to four air-impermeable polyethylene bags (Koch vacuum bag). To minimize sampling error, each bag was arranged to contain three pieces from different portions of the fish. The oxygen content in the CO_2-enriched packs was evacuated before introduction of carbon dioxide. All packs were tightly sealed and stored at 1 - 2°C in the dark. Three frozen fish also were thawed, cut, distributed into four similar polyethylene bags, and stored at −20°C in the dark. At 1, 6, 11, and 14 days of storage, one pack of each treatment group was opened for chemical analyses and sensory evaluation. The skin, bones and connective tissues were trimmed from the trout pieces, leaving only flesh portions for analysis.

Quantitative an qualitative analysis of astaxanthin

Homogenized trout flesh (approximately 20g) was extracted by a modified method of Kelley and Harmon (1972) to determine carotenoid content. The visible absorption spectra of the lipid residues were recorded on a Beckman 25 spectrophotometer. The total pigment concentration was calculated at the absorption maxima (467 - 470 nm) and the specific extinction coefficient ($E_{1\ cm}^{1\%} = 2400$) in petroleum ether was used (Kanemitsu and Aoe, 1958). For qualitative analysis, pigment extracts were concentrated and separated by column chromatography. A 20-cm column was packed under vacuum with an absorbent of Microcel-C (particle size <0.1 μ, Johns-Manville). Carotenoid pigments were eluted with a gradient of 5 - 10% acetone in petroleum ether (Kuo et al., 1976). Each collected fraction was evaporated and redissolved in petroleum ether and acetone for spectrophotometric analysis. The concentrate of each fraction also was identified by thin-layer chromatography. The pre-coated, 250μ layer, 20 x 20 cm Silica Gel plates were activated at 100°C for 1 hr. A solvent system of benzene-ethyl ether-methanol (17:2:1) was used (Noel, 1982).

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2-Thiobarbituric acid (TBA) analysis

The method of Vyncke (1970) was adopted for measurement of oxidative rancidity in the trout samples. Twenty-five grams of fish flesh were used for analysis. The TBA value, expressed as mg malonaldehyde/kg sample, was calcualted by the method described by Sinnhuber and Yu (1958).

Sensory evaluation

Seven panelists were selected and trained during which time various hues of raw (red to orange-red) and cooked (pink) salmon flesh were used for reference. The nine-point hedonic scale with five descriptive scales described by Ostrander et al. (1976) was adapted for evaluation of sensory properties, including visual color, texture, moistness, and flavor of samples. The highest scales for all the attributes were ranked from 9, except that 7 was the highest for the color of cooked fish with the elimination of pale salmon color. Trout flesh, approximately 3×2 cm^2, was wrapped individually in aluminum foil, baked in a commercial oven at 350°F (176.7°C) for 20 min, and cooled for 10 min before serving. Three replications for raw and cooked samples were prepared. The fillets, identified by random coding, were placed on a white chinaware plate for contrast.

The sensory evaluation was conducted under cool fluorescent illumination. Acceptability also was used as the criterion for evaluation of the 14-day-old samples.

Statistical analysis

All data were subjected to analysis of variance using a General Linear Models (GLM) procedure to study differences in pigment stability and sensory attributes of fish between packaging treatments (Freund and Littell, 1981). Correlation between the visual color scores and quantitative pigment content determination also was analyzed. The calculated F value exceeding 5% tabular F was considered evidence of a significant effect ($P < 0.05$).

RESULTS & DISCUSSION

THE FOUR PACKAGING TREATMENTS, i.e., air, oxygen-evacuated and CO_2-enriched packs ($1 - 2$°C), and air pack (-20°C) are designated as AR, $-O_2$, $+CO_2$, and AF, respectively, in the following sections.

Stability of the deposited carotenoids

The carotenoid pigment in the trout flesh was identified mainly as astaxanthin (free or esterified), with the composition profile shown in Table 1. The pigment content analyzed for all treatment groups at 1, 6, 11, and 14 days storage is illustrated in Table 2. Statistical analysis reveals a significant difference ($P < 0.05$) in pigment stability among packaging treatments. The pigment concentration in the trout flesh of 1 day old AR samples (9.8 µg astaxanthin/g tissue homogenate) was significantly higher than that reported by Ugletveit (1974) to be required for satisfactory pink-red color in rainbow trout ($2 - 3$ µg/g) or that stated by Spinelli and Mahnken (1978) in pen-reared coho salmon ($3.5 - 4.5$ µg/g). The highest pigment retention (ca 90%) was observed in AR samples during the 14-day storage period. In contrast, only 53% of the deposited astaxanthin remained in the AF samples. Higher color retention observed in air-packed ($1 - 2$°C) samples might be attributed to astacene and other breakdown products of astaxanthin. The

lower initial astaxanthin content in AF samples were due to the fish examined having been frozen for 3 days prior to packaging, during which color fading had occurred. A pronounced decoloration also was observed in the $+CO_2$ samples, wherein, degradation took place primarily within the first 6 days of storage. It is speculated that higher initial CO_2 concentrations in the pack could have reacted with haem pigments of the fish, as in the case of formation of metmyoglobin, and interfered with the determination of the flesh color (Parkin and Brown, 1983).

In the preliminary observations, a faintness in color was observed in unpacked trout fillets stored on ice overnight at 4°C. According to the results of the current investigation, normal packaging with an air-impermeable polyethylene bag was effective in short-term preservation of red coloration of pigmented trout at lower refrigeration temperatures ($1 - 2$°C). Temperature of storage and oxygen concentration are considered important in determining the rate of loss of carotenoid pigments during processing and storage of seafood products (Lusk et al., 1964). Nevertheless, as seen in the $-O_2$ samples, removal of the oxygen content of the packs did not improve pigment stability. Therefore, it is possible that an internal factor, i.e., endogenous enzymes, other than storage conditions per se, is involved in pigment degradation.

In a series of studies on the discoloration of red fish, Tsukuda and Amano (1967) observed that a lipoxygenase-type enzyme present in the skin tissue could convert astaxanthin into colorless compounds, i.e., carbonyl compounds, at refrigeration temperatures in the dark. The activities of this enzyme were affected by heat treatment, the nature and quantity of lipid components, and particularly the freshness of the fish. However, the enzyme was insensitive to freezing, and frozen storage as low as -30°C did not retard the discoloration process (Tsukuda and Amano, 1966).

Accordingly, TBA values were used to measure oxidative rancidity as well as the freshness of the fish studied (Kolakowska and Deutry, 1983). Based on TBA values (Fig. 1), the air-packed refrigerated samples deteriorated at a much faster rate than did other treatments. After one week of storage, the TBA values of AR samples were higher than 1 mg malonaldehyde/kg of sample, which is considered to be an indication of onset of oxidative rancidity in fresh fish (Sweet, 1973). As noted, the discoloring activities of the enzyme in the skin homogenate decreased as the freshness of fish declined, and complete inactivation occurred in the spoiled tissue (Tsukuda and Amano, 1968). In this regard, the greater stability of astaxanthin in AR samples may be due to the lower discoloring activities of the tissue enzyme resulting from the higher deterioration rate. As mentioned earlier, higher TBA values of AR samples might also substantiate the possible presence of astacene in the pigment extract. Details of mechanisms involved in the discoloration of red fish need further investigation.

Sensory evaluation and color assessment

The visual color scores of raw trout fillets for all treat-

Table 1—Analysis of deposited carotenoids in trout flesh on a diet containing 45 mg astaxanthin/kg feed

Fraction	R$_f$ value	Absorption maxima (nm)	Solvent	Pigment identification	% Composition
1	0.85 – 0.85	465 477	petroleum ether acetone	astaxanthin ester	41.7
2	0.40 – 0.45	467 480	petroleum ether acetone	astaxanthin	58.3

ments are presented in Table 3 with a significant difference (P < 0.01) observed among treatments. These scores were consistent with data on astaxanthin contents analyzed (Table 2), with a correlation coefficient of r = 0.77 (P < 0.001) being observed. In a study dealing with color assessment of experimentally pigmented rainbow trout, Little et al. (1979) indicated that measurements relating to specific absorption characteristics of astaxanthin pigment also relate directly to visual perception. However, in their study, it was observed that pigment concentration alone could not serve as a predictor of color. Both reflectance measure-

ments, i.e., colorimetry and visual appearance, were influenced significantly by differences in properties of light-scattering cellular matrix at constant pigment concentration.

The scores of other sensory attributes of raw and baked trout fillets are shown in Table 4. A significant difference (P < 0.01) in visual color of cooked samples was noted among treatments. During cooking, a shift occurred in hue from red to pink, giving a lighter and less pure color. The resultant pinkish red color of cooked trout indicated that the astaxanthin-pigmented fish, obtained by feeding a diet containing crawfish pigmented oil extract, retains color satisfactorily under normal heat treatment. Statistical analysis of other sensory attributes showed a significant difference in moistness (P < 0.05) and texture (P < 0.005) of raw trout fillets among treatments. The arithmetic mean data indicated that AF raw samples had the highest moisture scores, while AR raw samples retained maximum firmness. The +CO$_2$ samples had the lowest texture scores in the fresh trout examined. The softening of tissue in the fillet may be due to the higher CO$_2$ concentrations (Parkin and Brown, 1982). No differences were seen in flavor, moistness, and texture attributes of cooked fillets among treatment groups. Except for minor spoilage occurring in the flesh near the skin areas of the 14-day-old AR samples, the acceptability of all raw and cooked trout samples remained good throughout the test period.

In an ancillary experiment, good color retention was observed in frozen packs of pigmented rainbow trout fillets stored for a month. Accordingly, it is evident that freezing of fish fillets, with heading and skinning, packed in air-impermeable polyethylene bags can be used for proper preservation of the freshness and coloration of pigmented rainbow trout.

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Fig. 1—TBA values of trout fillets.

Table 2—Stability of deposited carotenoids in trout flesh

Treatment	Pigment concentration (μg/g) Storage period (day)			
	1	6	11	14
AR	9.8 ± 0.8	9.5 ± 0.9	9.0 ± 1.0	8.8 ± 1.0
−O$_2$	9.0 ± 0.9	6.8 ± 1.0	6.2 ± 1.1	5.9 ± 1.2
+CO$_2$	7.8 ± 1.1	6.6 ± 1.4	6.2 ± 1.3	5.5 ± 1.6
AF	6.8 ± 0.2	6.5 ± 0.8	5.6 ± 1.6	5.2 ± 1.1

Table 3—Sensory color scores of raw trout fillets

Treatment	Storage period (day)			
	1	6	11	14
AR	8.9 ± 0.5	8.8 ± 0.7	8.8 ± 0.4	8.7 ± 0.5
−O$_2$	8.6 ± 1.1	8.5 ± 1.2	8.5 ± 0.7	8.3 ± 1.0
+CO$_2$	8.1 ± 1.0	7.7 ± 1.6	6.7 ± 0.9	6.7 ± 0.7
AF	7.2 ± 0.7	6.7 ± 1.3	6.2 ± 1.7	6.2 ± 1.2

Table 4—Sensory scores of raw and cooked trout fillets.

Treatment	Storage period (day)	Color Cooked	Texture Raw	Texture Cooked	Moisture Raw	Moisture Cooked	Flavor Cooked
AR	1	6.3	7.8	6.4	6.2	6.1	5.3
	6	6.1	8.3	6.3	5.3	5.3	5.7
	11	6.3	7.6	7.0	6.4	5.7	4.0
	14	6.5	8.0	6.4	6.6	5.8	4.8
−O$_2$	1	5.8	6.2	6.3	6.5	6.0	5.4
	6	5.8	7.3	6.3	5.3	5.3	4.5
	11	6.1	7.7	7.0	5.9	5.4	5.0
	14	6.0	7.6	6.5	6.3	5.5	5.0
+CO$_2$	1	5.3	7.3	6.7	6.8	6.7	5.0
	6	5.2	7.3	6.5	5.5	5.3	4.7
	11	4.8	6.7	7.7	6.7	5.2	4.5
	14	5.0	6.8	6.5	6.5	5.1	4.1
AF	1	5.0	7.2	5.7	7.0	6.2	5.2
	6	5.3	6.7	6.7	6.5	5.8	5.2
	11	5.0	7.0	7.0	7.0	4.8	5.2
	14	4.9	6.6	6.6	7.2	5.7	4.7

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NONENZYMATIC BROWNING IN PEAR JUICE ...

Table 2—Formol titration of pear juice concentrate held at 80°C, measured as glycine in mg/100 mL/°Brix

°Brix	Heating time (hr)	
	0	4.1
45.2	5.10[a]	5.32[a]
55.4	5.56[a]	4.43[b]
65.1	5.20[a]	4.29[b]
72.5	4.91[a]	3.96[b]

[a,b] Means bracketed by the same letter are not significantly different (P ⩾ 0.05).

predicted as a browning rate at 37°C for the present d'Anjou pear juice concentrate. Clearly, this value does not agree with literature estimates, but equally clearly the literature estimates do not agree with each other. This would suggest that caution is appropriate when considering extrapolation of present results to longer term storage at lower temperature. The possible effects of pear variety, harvest condition or agronomic practice on browning rates are not known. Any of these factors could affect the levels of free amino acids or their distribution in the juice. Either could have a marked effect on the degree of browning experienced with concentrate prepared from that juice.

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Effect of Some Potentially Synergistic Treatments in Combination with 100 Krad Irradiation on the Iced Shelf Life of Cod Fillets

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―――――――― ABSTRACT ――――――――

Irradiation of cod fillets with a maximum absorbed dose of 100 Krad extended iced storage life by about 9 days. Further extension of several days resulted when irradiation was combined concurrently with either 60% CO_2 packaging atmosphere or sorbate additive. Packaging at low oxygen tension did not provide any additional benefit for irradiated fish. No important difference in storage life of treated fish was observed due to one vs three day postmortem age. The 100 Krad treatment extended grade B quality market life as opposed to grade A (prime quality) market life. Certain physical/ chemical tests were evaluated for their efficacy in estimating spoilage. Concentrations of TMA, DMA, hypoxanthine, APC and pH at spoilage were comparable in control and air-irradiated samples, but were less in sorbate-irradiated fillets.

INTRODUCTION

RESEARCH in the United States on preservation of foods by ionizing radiation dates back to the 1940's, yet the process has not been sanctioned by the FDA as a food preservation method.

In 1977 the joint FAO/IAEA/WHO Expert Committee on Wholesomeness of Irradiated Food gave provisional acceptance for irradiation of cod and redfish (ocean perch) up to a maximum dose of 220 Krad, and in the fall of 1980 that committee recommended unconditional acceptance of irradiated foods up to a dose of one million rads (10 kGy) (WHO, 1981). More recently an internal task force created by FDA to provide some recommendations for irradiation preservation based on recent findings and current state-of-the-art knowledge in toxicology and radiation chemistry, recommended that food irradiated at doses up to and including a maximum of 100 Krad (1 kGy) will be deemed wholesome and safe for human consumption (FDA, 1981). Of immediate concern to many seafood processors is the shelf life extension to be expected after treatment with a maximum dose of 100 Krad. It should be pointed out that due to the geometrical conformation of the product being irradiated, there will be a gradient in absorbed dose within the product. With gamma irradiation the maximum absorbed dose will be at the product surface closest to the irradiation source and the minimum will usually be either at the center or at the surface furthest from the source depending on the source configuration. In most published food irradiation studies the author(s) failed to state whether the applied dose was maximum, minimum or average. The shelf life extension of irradiated fish is a function of many variables including dose level, pre-irradiation quality, number and types of bacteria present, product environment and storage temperature. Thus, a simple answer as to the extension provided by a maximum dose of 100 Krad cannot be given unequivocally. Some shelf life extensions for various fish species after treatment with different dose levels under varying conditions are cited in a recent comprehensive review on preservation of seafood with ionizing radia-

tion (Nickerson et al., 1983). Miyauchi (1970) reported that cod fillets treated with 100 Krad and shipped on ice across the country by commercial carrier had a shelf life extension of one week. If this is typical, some processors may feel that a shelf life extension of one week on ice may not justify the added cost of irradiation. Hannesson and Dagbjartsson (1970) recommended an irradiation dose of 200 Krad in preference to 100 Krad for cod because it gave a longer extension of shelf life required to deliver irradiated fish to foreign markets. A longer shelf life would also be desirable for the application where fish would be irradiated and then stored in a central warehouse with controlled release to distribution channels for the purpose of stabilizing market supply. Cod can withstand irradiation treatment in excess of 100 Krad without having incurred any undesirable sensory changes. The maximum acceptable dose level for cod has been reported to range from 150 – 300 Krad (Ampola et al., 1969; Rhodes, 1964; Shewan and Liston, 1958).

Certain packaging or chemical treatments have been shown to enhance shelf life of fresh fish. These processes include modified atmosphere storage (Killeffer, 1930; Stansby and Griffiths, 1935; Martin, 1981), sorbate treatment (Debevere and Voets, 1972; Shaw et al., 1983) and vacuum packaging (Shewan and Hobbs, 1963; Huss, 1971). It is known that some process treatments applied concurrently with irradiation exert a synergistic effect. These include the use of thermal energy (Licciardello, 1964; Goldblith and Nickerson, 1966), antibiotics (Lerke et al., 1961; Awad et al., 1965; Tomiyama et al., 1969) and chemical preservatives (Shehata, 1961; Thornley and Ingram, 1963; Lee et al., 1965). The combined effect of sorbate treatment and ionizing radiation was studied in previous years, but the purpose of the sorbate was to suppress yeasts which eventually cause spoilage in foods irradiated at doses of about 500 Krad. Vacuum packing has been combined with irradiation, but mainly to inhibit oxidative rancidity. Irradiation and CO_2 atmosphere storage was applied by Graikoski et al (1968) to yellow perch fillets, but as a two step process.

The primary purpose of this investigation was to determine whether the iced shelf life of fish fillets irradiated with a maximum dose of 100 Krad could be extended by combining the irradiation treatment with one of these ancillary processes. A secondary objective was to determine the efficacy of certain conventional physical/chemical spoilage tests for assessing quality or spoilage of fillets treated with the combined irradiation processes since a possible shift in the spoilage microflora might preclude the reliability of these tests.

MATERIALS & METHODS

THIRTY COD FISH (8 – 10 lb each) of one day postmortem age were obtained from a Gloucester, MA day boat and divided into two lots. One lot was iced for continued storage and the other was filleted for immediate experimental use. Six fillets (skin-on) were individually sealed in an air atmosphere in Curlon S-660 (nylon-PVDC-surlyn) pouches and served as non-irradiated controls. The remaining 24 fillets were apportioned into four lots from which single fillets were sealed: 1) in an air atmosphere; 2) under vacuum

using a Kenfield Model C-14 vacuum sealer; 3) in an atmosphere (slight positive pressure) of 60% carbon dioxide-40% air; 4) dipped 40 seconds in five percent potassium sorbate solution (2 parts solution/1 part fish v/w) prior to packaging. All were irradiated in the Marine Products Development Irradiator (cobalt-60) at the Gloucester Laboratory to a maximum absorbed dose of 100 Krad. Dose distribution within the mass of fillets was determined with ferrous-cupric sulfate dosimeters (Hart and Walsh, 1954) placed at various locations and was found to be 1.3 to 1. Thus, when the fillets were irradiated to receive a maximum dose of 100 Krad, some sections within the mass only received about 75 Krad. Following irradiation all samples were stored in ice and monitored for quality loss and onset of spoilage by sensory, instrumental, mcirobiological and biochemical tests (Lima dos Santos, 1981).

After a two-day additional iced storage (three day postmortem), the second lot of fish was similarly treated.

Sensory analysis

Each fillet was evaluated in the raw state for appearance and odor, and in the cooked state for flavor and texture by a six member panel made up from the scientific laboratory staff experienced in judging cod. Samples were rated on a scale ranging from 9 = excellent to 1 = inedible and were considered unacceptable when the average score for any of the sensory attributes fell below 5.5. For sensory evaluation of the cooked product, the fillets were placed in a foil-covered pan and cooked in live steam (100°C) for 12 - 15 min. Each panelist was served an approx. 50g portion. A frozen control was included as a reference sample.

Torrymeter

Readings were taken with a GR Torrymeter (Jason and Richards, 1975) at three different positions on the fillet along the lateral line, at the head end, at a central position below the second dorsal fin, and at the tail end. Six measurements were made in each location and the results averaged.

pH

A 20-g composite sample of muscle from various sections of the fillet was blended with 40 mL distilled water for 1 min and the pH of the slurry was measured with a Fisher Model 320 expanded scale pH meter.

DMA AND TMA

Dimethylamine (DMA) and trimethylamine (TMA) analyses were performed by a gas chromatographic method (Lundstrom and Racicot, 1983). The amines were extracted first with perchloric acid, then with n-amyl alcohol after neutralization by KOH, separated on a porous polymer column (Chromosorb 103) and identified using a N/P Specific Flame Ionization Detector.

Hypoxanthine

The enzymatic procedure described by Jones et al (1964) was employed for the determination of hypoxanthine.

Aerobic plate count

The aerobic plate count (APC) was made from appropriate dilutions onto pour plates of TPE agar (Standard Methods Agar reinforced with 0.5 percent Bacto-peptone and 0.5% NaCl) as recommended by Lee and Pfeifer (1974) for seafoods. Duplicate plates were incubated at 20°C and colony counts were made after 5 days.

Statistics

Analyses were performed in duplicate and results were averaged. Statistical analyses for linear regression, correlation, analyses of variance and multiple range test (Duncan, 1955) were conducted on programmed calculators, either the HP 97 or HP 41CV.

RESULTS & DISCUSSION

INDEPENDENT RESULTS with 1- or 3-day postmortem fish indicated no major difference in shelf life due to pre-irradiation quality. Therefore, the data from the two experiments were compounded for simplification and these combined results will be reported.

The raw odor scores for the irradiated and nonirradiated fillets following various storage intervals in ice are shown in Fig. 1. The controls were significantly different (1% level) in odor from the treated irradiated samples after 10 - 11 days but not after 6 days. No significant difference in odor among irradiated samples was observed until the 27th day at which time the sorbate-irradiated treatment was significantly different (5% level) from either air-irradiated or vacuum-irradiated samples, but not from the 60% CO_2/ 40% air irradiated samples. The transition from grade A to grade B of well-iced cod usually occurs after 5 - 7 days storage. This event coincided with the time at which the samples were rated as "good" or score = 7. Thus, although it is conceded that the various low-dose irradiation treatments extended the iced shelf life, it appears that the grade B quality life of the fillets was principally extended and not the grade A prime quality. From studies with haddock or ocean perch irradiated with 100 Krad, Ehlermann and Reinacher (1978) also concluded that low dose irradiation treatment only extended the shelf life during which the product was at a grade below high quality.

The rates of flavor deterioration during storage generally paralleled those of odor deterioration (Fig. 2). The nonirradiated controls were significantly different (1%

Fig. 1—Effect of iced storage on the raw odor score of cod fillets either non-irradiated or irradiated (100 Krad) under various conditions.

Fig. 2—Effect of iced storage on the flavor score of cod fillets either non-irradiated or irradiated (100 Krad) under various conditions.

level) from the treated irradiated samples after 19 – 20 days, but not after 10 – 11 days. No significant flavor score difference was evident among the treated irradiated fillets until the 27th day at which time the sorbate-irradiated treatment was significantly different (1% level) from either the air- or vacuum-irradiated treatment, but not from the CO_2 irradiated treatment. Vacuum packaging did not enhance flavor stability of the irradiated samples during storage. Ampola et al. (1969) had reported that vacuum packaging of cod fillets irradiated in the range of 150 – 350 Krad was detrimental to the flavor, but we did not observe that effect. Vacuum packaging of seafood to be irradiated has generally been found to be beneficial, regarding flavor stability, to those fish species susceptible to the development of oxidative rancidity (Nickerson et al., 1983). Iced cod is not in this category.

The total iced shelf lives for any of the treatments based on the time at which either the raw odor score, cooked flavor or texture score reached a rating of 5.5 were estimated from linear regression analysis (sensory score as a function of storage time) as follows: control = 10 days, air
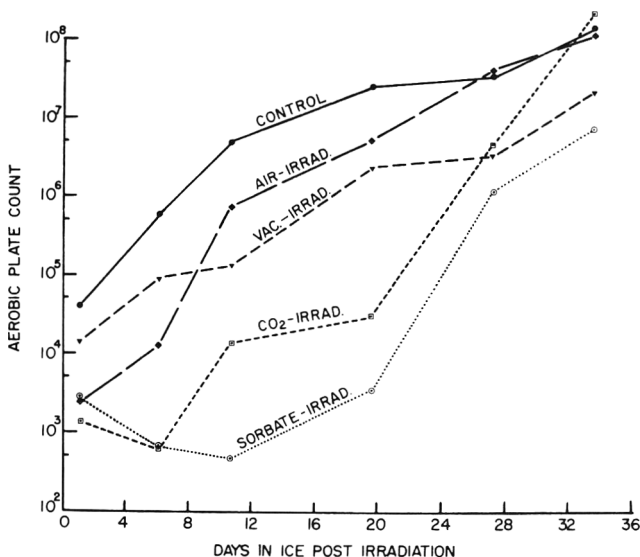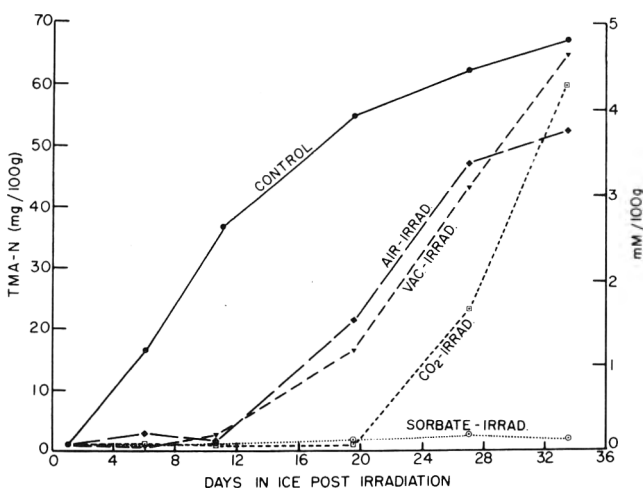
irradiated = 19 days, vacuum irradiated = 21 days, CO_2 irradiated = 24 days, sorbate irradiated = 25 days. These shelf lives represent the elapsed time from harvest to end of acceptability assuming an average post mortem age of two days. Raw odor was usually the limiting quality factor governing shelf life. The results suggest that the greatest shelf life extension resulted from either the sorbate-irradiated or CO_2 irradiated treatments.

The rates of bacterial (APC) growth in the variously treated samples can be seen in Fig. 3. Except for the vacuum packed samples, the irradiation treatments reduced the initial bacterial concentration by a factor of 90 – 95 percent or just more than one log cycle. A lesser reduction in microbial concentration occurred in vacuum packed samples. That microorganisms are less sensitive to the effects of ionizing radiation in the absence of oxygen is well known (Goldblith, 1963). The subsequent rate of growth in the treated samples more or less paralleled that of the control, but in the case of the CO_2-irradiated and especially the sorbate-irradiated samples, there was an extended lag phase which accounted for the increased shelf life. An APC of 1 $\times 10^6$/g has usually been employed in studies with fresh fish to indicate incipient spoilage. The number of days to reach this count in the various treatments was as follows: control, 8-9; air-irradiated, 15; vacuum-irradiated, 19-22; CO_2-irradiated, 23-28, sorbate-irradiated, 27-33. These times are in good agreement with storage lives estimated from sensory evaluation. From the microbiological results it would appear that the sorbate-irradiation treatment provided the longest shelf life.

Trimethylamine content increased steadily in control samples from the first day of storage whereas in irradiated samples there was a delay of at least 10 days before significant amounts began to be detected (Fig. 4). With CO_2-irradiated samples the lag period extended to about 20 days and in sorbate-irradiated samples there was practically no formation of TMA over a 34 day storage period. Both sorbate-irradiated and CO_2-irradiated treatments retarded DMA development whereas the rate of formation in air-irradiated or vacuum-irradiated samples was not much different from controls (Fig. 5). Spoilage of seafoods at temperatures above freezing is usually due principally to bacterial action and in this regard some microbial species, particularly the pseudomonads, play a more important role compared to others (Shewan, 1961). However, of the various bacterial species normally present on fish from temperate waters, the pseudomonads are probably the most radiosensitive (Thornley, 1963) and their numbers are reduced to a very low level after treatment with even a low dose of ionizing radiation. If the pseudomonads are

Fig. 3—Aerobic plate count of non-irradiated or treated-irradiated (100 Krad) cod fillets as a function of storage time in ice.

Fig. 4—Trimethylamine-nitrogen content of nonirradiated or treated-irradiated (100 Krad) cod fillets during storage in ice.
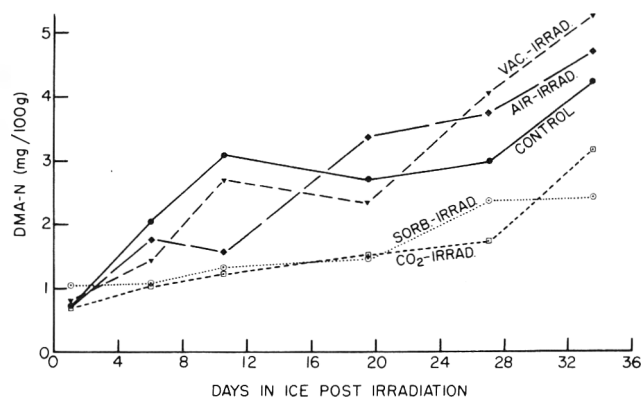
Fig. 5—Dimethylamine-nitrogen content of nonirradiated or treated-irradiated (100 Krad) cod fillets during storage in ice.

completely eliminated, either through application of a higher irradiation dose or through some other inhibitory treatment, then spoilage will be caused by the metabolic activity of some other bacterial species and an unconventional spoilage pattern may develop (Corlett Jr., 1967). Since most of the biochemical tests for fish quality are based on the concentration of certain chemical compounds such as TMA resulting chiefly from the metabolic activity of certain pseudomonads, then these quality tests may not be reliable when these pseudomonads have been eliminated.

Hypoxanthine (Hx) concentration increased at a steady rate in control samples reaching a maximum after about 27 days (Fig. 6). The Hx levels in most irradiated samples also peaked at about 27 days. A major difference in Hx content between control and irradiated treatments was apparent from the tenth day on. Hx formation in fish during the early post-mortem period has been attributed mainly to autolytic enzyme action; in the later storage stage bacterial action plays a role (Jones, 1965; Burt, 1976). The correlation coefficient for Hx as a function of log APC was 0.80 for the control and 0.87 – 0.91 for the irradiated treatments, inferring that bacterial action played an important part in Hx formation. Parris et al. (1983) demonstrated that Hx formation in refrigerated beef was due to endogenous enzymes and not to microbial activity.

Torrymeter readings taken on the skin of the fillets during storage are shown in Fig. 7. The more rapid decrease in meter reading during storage of the control was consistent with the rates of sensory quality loss in irradiated and non-irradiated samples. Although there were slight differences in the rate of decrease of the meter reading among the various irradiation treatments during storage, no major quality difference was discerned with the meter. There was good correlation between raw odor score and meter reading, ranging from 0.70 – 0.88 for all treatments.

The irradiation effected an initial lag in pH development, but following this lag the rate of increase in alkalinity was similar to the nonirradiated control (Fig. 8). There was essentially no change in pH in sorbate-irradiated samples and just a slight increase in pH in the CO_2 irradiated treatment over the 27 day post-irradiation period. The unchanged pH of sorbate-irradiated samples during storage was not attributed to the sorbate because the dip solution was alkaline. The development of a high final pH in the vacuum-irradiated sample would preclude the emergence of a lactic acid-producing microflora as has been found under conditions of vacuum packing with low dose irradiation

in previous investigations (Pelroy and Eklund, 1966; Licciardello et al., 1967).

Data from the various biochemical, instrumental or microbiological analyses were correlated with raw odor scores and from the regression line the value signalling terminal acceptability was predicted from an odor score of 5.5. These values are given in Table 1; the correlation coefficients are shown in parenthesis. Aerobic plate counts at spoilage for all treatments ranged from $0.4 – 1.8 \times 10^6/g$. An APC of approximately $10^8/g$ is usually observed at spoilage for low dose irradiated fish (Spinelli et al., 1969), but when that occurs the product is usually devoid of pseudomonads and the spoilage pattern is of a nonproteolytic nature. The irradiation dose required to eliminate pseudomonads from fish depends on the initial concentration of these bacteria, and ranges from 100 – 200 Krad. Laycock and Regier (1970) reported that a greater percentage of pseudomonads survived a dose of 100 Krad in nine day compared to 2 day postmortem haddock fillets, and the spoilage flora of the nine day fillets contained a higher proportion of pseudomonads. Although the spoilage microflora of low dose irradiated fish has usually been found to consist principally of achromobacter (moraxella and acinetobacter), reports of spoilage by pseudomonads have been published (Shewan and Liston, 1958; Pelroy and Eklund, 1966).

The TMA-N level in the control samples at spoilage was estimated to be 28 mg/100g. This spoilage level for cod is in agreement with the value of 30 mg/100g proposed by Hillig et al. (1962), but not with levels suggested by others,
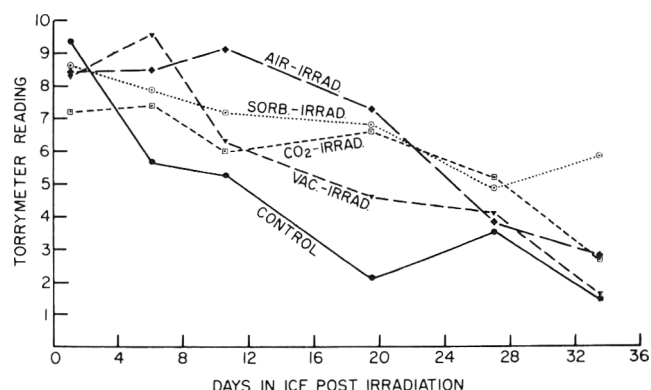
Fig. 7—Torrymeter readings during iced storage of cod fillets either nonirradiated or irradiated (100 Krad) under various conditions.
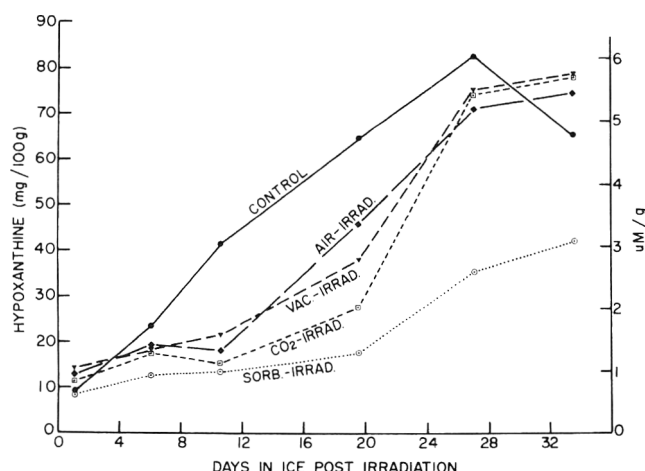
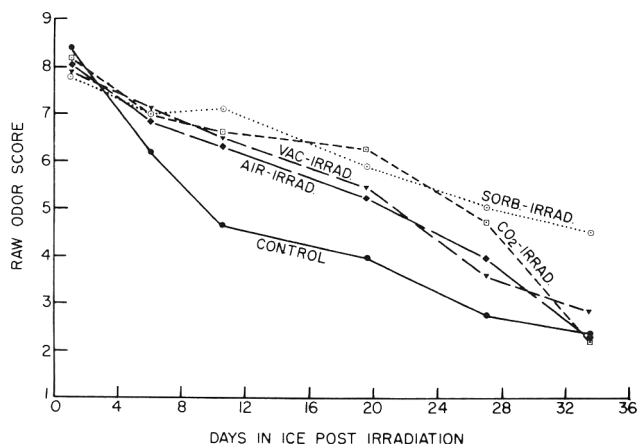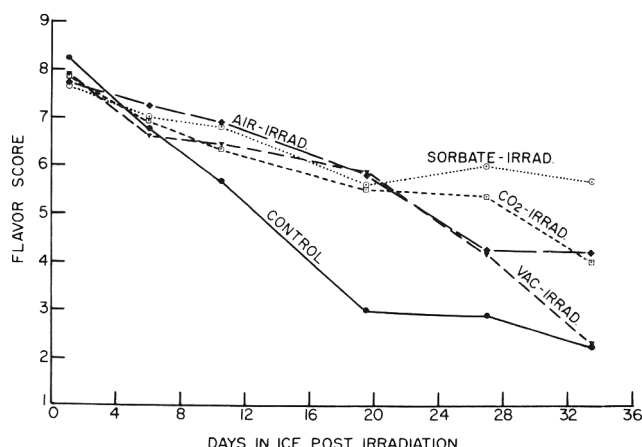*Fig. 6—Hypoxanthine content during iced storage of cod fillets either nonirradiated or irradiated (100 Krad) under various conditions.*

Fig. 8—Effect of iced storage on pH of cod fillets either nonirradiated or irradiated (100 Krad) under various conditions.

Table 1—Numerical values of various biochemical, instrumental or microbiological parameters for treated cod fillets predicted from an odor score of 5.5. (Correlation coefficient in parenthesis)

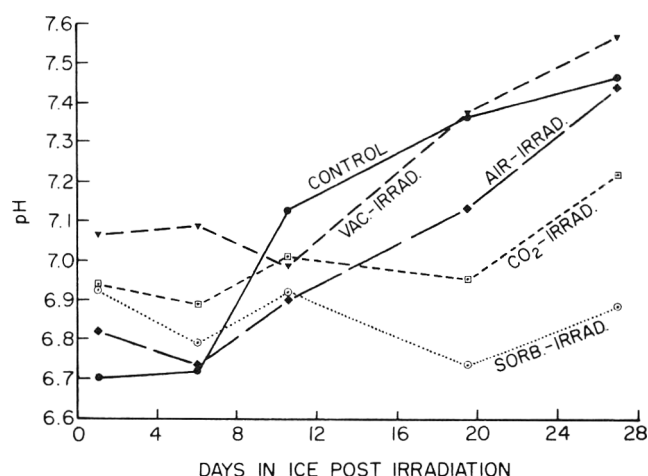| Treatment	APC	TMA-N mg %	DMA-N mg %	Hx u M/g	Torrymeter	Muscle pH
Control	1.8×10^6 (0.96)	28 (0.96)	2.4 (0.71)	2.9 (0.87)	5.4 (0.88)	7.02 (0.89)
Air-irradiated	1.1×10^6 (0.92)	18 (0.90)	2.6 (0.89)	2.9 (0.90)	6.9 (0.83)	7.10 (0.91)
Vacuum-irradiated	0.7×10^6 (0.90)	19 (0.91)	2.7 (0.83)	3.0 (0.94)	5.6 (0.85)	7.40 (0.87)
CO_2-irradiated	0.4×10^6 (0.91)	15 (0.34)	1.8 (0.82)	3.1 (0.90)	5.5 (0.72)	7.15 (0.89)
Sorbate-irradiated	0.8×10^6 (0.73)	1.6 (0.54)	1.7 (0.71)	2.0 (0.82)	6.3 (0.68)	6.86 (0.10)

for example: 7 mg/100g (Hoogland, 1958), 6 – 12 mg/100g (Reay and Shewan, 1949), 15 mg/100g (Dyer and Dyer, 1949) and 10 – 20 mg/100g (Dyer and Mounsey, 1945). The discrepancies may be due to methodology, sampling, or discrimination among different sensory panels regarding the quality level of the fish constituting spoilage. Dyer and Dyer (1949) reported TMA-N levels in iced cod of 30 and 40 mg/100g after 12 and 13 days respectively. Our values agree with theirs, which suggests TMA formation was similar in both cases, yet those authors proposed the value of 15 mg/100g as the index of spoilage. Less TMA was present in irradiated samples at spoilage with the least amount in the sorbate treated samples. The suppression of TMA production in iced fish following low dose irradiation has been reported (Shewan and Liston, 1958; Power et al., 1964). Miyauchi (1960) observed a TMA-N content of only 4.4 mg/100g in iced, irradiated (100 Krad) Pacific cod fillets after 12 wk, even though the fillets were considered unacceptable. Spinelli et al. (1969) related TMA content at spoilage with irradiation dose and concluded that TMA as a quality index is only useful in fish irradiated below 200 Krad because of the very small amount formed at higher doses. Laycock and Regier (1970) have shown that TMA production in irradiated fish is not only a function of the dose, but also of the pre-irradiation quality of the fish. Following irradiation at 100 Krad, TMA was produced at a higher rate in 9 day compared to 2 – 5 day postmortem haddock fillets. TMA would not be a useful indicator for spoilage in sorbate-irradiated cod because of the total inhibition of its development, but with the other irradiation treatments a sufficient amount was formed to assign spoilage limits.

DMA-N concentration was about the same (2.4 – 2.7 mg/100g) for control and air-irradiated or vacuum-irradiated samples, and slightly less (1.7 – 1.8 mg/100g) for CO_2 or sorbate irradiated treatments at the point of unacceptability. Unlike TMA formation, which had a lag of several days before an appreciable increase was detected, DMA increased at a relatively constant rate during immediate postmortem storage. DMA analysis was proposed by Reay and Shewan (1949) as a test for measuring early quality changes in gadoid fish. We were not able to find a proposed spoilage level in the literature. With reference to data shown by Reay and Shewan (1949), at their proposed spoilage stage of 6 – 12 mg/100g TMA-N, the corresponding DMA-N concentration was about 1 mg/100g. Yet, when the TMA-N reached a level of 28 mg/100g, observed at spoilage in our study, the associated DMA-N concentration was about 2.8 mg/100g, agreeing with our DMA-N spoilage level. This seems to indicate a difference in sensory criteria for spoilage and not a difference in analytical results or DMA accumulation. Dyer and Mounsey (1945) reported that fresh cod contained about 0.1 mg/100g DMA-N which increased to about 3 – 4 mg/100g in spoiled fish. Because of the variable results they concluded the test was unreliable as a spoilage indicator and we would tend to agree.

There was very good correlation between raw odor score and Hx content. Jones (1965) had also observed that flavor scores in cod fell at a rate nearly directly proportional to the increase in Hx content. Hx levels at spoilage for all treatments based on odor score ranged from 2 – 3 µM/g. Burt et al. (1969) assigned a value of 2.5 µM/g for cod at rejection. Except for the sorbate irradiated treatment, this value is slightly higher than that determined in the present study. We agree with Spinelli et al. (1969) that the Hx test can be used to measure quality changes in low dose irradiated fish.

Although Torrymeter readings for unacceptability ranged from 5.4 – 6.9 for the various treatments, they were not considered to be different in view of the magnitude ($\sim$1.5) of the associated standard errors. It is not expected that Torrymeter readings for iced cod or haddock of the same postmortem age would differ and in a previous study with iced haddock, a Torrymeter reading of 6 was indicated at the time of rejection (Licciardello et al., 1980). The results of the present study are compatible with this earlier finding. It is considered that the Torrymeter could be used to assess spoilage in low dose (100 Krad) irradiated fish provided the instrument is used cautiously, that is, sampling a sufficient number of fish, taking readings in the same general location, and avoiding measurements on bruised skin surfaces.

Muscle pH at spoilage was slightly lower in the control compared to irradiated samples except for the sorbate irradiated treatment in which there was virtually no pH change during storage. Therefore, this parameter would not be useful in assessing spoilage for this treatment. The variability in pH among the other treatments would make pH an uncertain quality test.

In summary, irradiation of cod fillets with 100 Krad under various conditions extended the period of acceptability, but not so much the period of high quality during iced storage. On the basis of quality (acceptability) as measured by certain chemical or physical tests, sorbate-irradiated treatment provided the longest shelf life. When assessed by sensory methods the sorbate-irradiated or CO_2-irradiated treatments were equally effective in maximizing shelf life.

A major concern about the use of process treatments that extend refrigerated shelf life of foods is the possibility of growth and toxin production by C. botulinum before the product becomes recognizably spoiled. Storage at temperatures below 3°C will preclude that situation; however, one cannot guarantee against temperature abuse once a

product enters the distribution channel. There has been recent evidence to indicate that potassium sorbate inhibits spore germination, cell growth and toxin production by *C. botulinum* (Smoot and Pierson, 1981; Roback and Sofos, 1982; Blocher and Busta, 1983). In inoculated studies with whitefish packed in a CO_2 atmosphere and held at $21^\circ C$, toxin production was delayed in fillets which had been dipped in potassium sorbate solution (Lindsay, 1981). Thus, treatment of fish to be radio-pasteurized with sorbate may provide a safety factor against botulinogenesis.

Packaging of fresh seafoods in modified atmospheres has also evoked the concern of some over the safety aspects from botulism and the industry has been advised to proceed cautiously with its application (Anon, 1981). With inoculated fillets packed in CO_2 atmospheres there was shown to be a definite botulism risk at high storage temperatures $21 - 27^\circ C$, a moderate risk at $10^\circ C$ storage, and no risk under good refrigeration such as $4^\circ C$ or lower (Lindsay 1981; Lindsay et al., 1982). Since the irradiation dose of 100 Krad would not effect a significant reduction in spore concentration of type E *C. botulinum* in fish fillets, it would have to be presumed that the botulism risk in CO_2-irradiated cod fillets would be similar to that in unirradiated CO_2-packed fish.

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Evaluation of Rapid Fat, Moisture, and Protein Determination in Meats and Meat Products using an Automatic Meat Analyzer

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———————— ABSTRACT ————————

The Hobart FMP-1 Automatic Meat Analyzer has been developed for rapid (approximately 5 min), simultaneous determination of the fat, moisture and protein content of meat and meat products. Results were compared with those using official AOAC methods for raw beef and pork and cooked sausage samples. Analyses of the data indicated that, except for determination of fat content of raw pork and protein content of raw beef, the Hobart FMP-1 was not significantly different from the official AOAC methods within the sampling parameters studied. From a practical meat operations standpoint, these differences were not considered to be of major concern as the machine can be calibrated to adjust for bias.

INTRODUCTION

A NUMBER OF PAPERS have been published dealing with the evaluation of newer, more rapid methods for determining the fat content of meat and meat products. Pettinati et al. (1973a and 1973b) have written reviews focusing on the savings in time and the relative accuracy of the new methods compared to conventional AOAC methods (1980). The accuracy and precision of the Foss-let fat analyzer was evaluated in depth by Pettinati and Swift (1975). Most evaluations of new analytical methods to the present have dealt with improvements in the fat analysis of food products. However, the Hobart FMP-1 Automatic Meat Analyzer (Hobart Corporation, Troy, OH 45374) has been developed for the rapid (approximately 5 min), simultaneous determination of the fat, moisture and protein content of meat and meat products.

The FMP-1 analyzer operates on the principle of using microwave energy to separate the fat and moisture from the protein and ash of a meat sample by uniformly heating the sample. The moisture is vaporized and released while the fat is melted and collected. The sample is cooked to a point short of the decomposition of the protein, while the remaining residue has a relatively constant chemical analysis. The fat, moisture and protein components of the product are computed through microprocessor monitoring of the weight loss of the sample and by subsequent calculations pertaining to the amount of moisture and fat separated from the meat sample.

As of the present time, no data have been published relative to the performance of the FMP-1 Analyzer in comparison to established AOAC methods.

The objective of this study was to determine the accuracy and precision of the FMP-1 Analyzer in comparison to AOAC methods (24.003, 24.005, and 24.027) for fresh and processed meats.

MATERIALS & METHODS

Apparatus

Hobart FMP-1 Analyzer (Hobart Corporation, Troy, OH 45374).
Soxlet, oven moisture and Kjeldahl methods used for the an-

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alyses of fat, protein and moisture, respectively, were as described in AOAC (1980).

Determination

Sample preparation. Fresh beef and pork cuts were prepared for analysis by initially grinding through a 5/8" plate and mixing by hand to obtain a uniform blend. The sample was ground through a 3/32" plate, mixed, using a kneading/folding action for 30 sec and ground a second time through a 3/32" plate and remixed. The beef and pork samples were prepared by mixing various proportions of lean and fat tissues to yield samples which covered a range of fat values (approximately 10–40%). Care was taken to maintain the final temperature of the prepared sample between 30° and 55°F. A 70–80g subsample was selected from the prepared sample blend for analysis in the FMP-1 Automatic Meat Analyzer.

National brand frank and bologna samples were purchased in retail markets for the cooked sausage product samples. These samples were prepared in an identical manner to the fresh meat samples, except the first grinding (5/8" plate) was omitted and the sample was ground one time through a 3/32" plate.

Analysis. The FMP-1 Analyzer was energized and the date, run number, meat type, percent salt by analysis (where necessary), and sample temperature were entered by engaging the appropriate panel digits. The oven was prepared for sample entry by loading a clean fat dish (with two sheets of dish paper and a watchglass) and sample holder onto the sample support in the oven. After the tare was set in the unit for the container and dishes, the sample holder was removed and the bottom portion of the holder placed into the base of the sample press. Seventy to eighty grams of meat sample were loaded into the sample holder, placed on the sample support in the oven, and the cooking process initiated. At the end of the cook cycle (2 – 4½ min), the final percent fat, percent moisture and percent protein results were displayed on both the LED panel and a paper printout.

Samples were statistically analyzed by beef, pork, and cooked sausage groups as well as by combining beef and pork. An overall-products category was also evaluated. Statistical data for each product group included: means for each method; means, standard deviations and range of the differences between methods; within lab repeatability by method; and coefficient of variation between methods. The t-statistic was calculated for a comparison of results between the Hobart and A.O.A.C. methods by group (Snedecor, 1956; Youden and Steiner, 1975).

RESULTS & DISCUSSION

FAT, MOISTURE AND PROTEIN determinations were conducted on seven beef, six pork, and twelve cooked sausage (bologna and frankfurters) product samples using the FMP-1 Analyzer and AOAC methods. The results of the analyses for fat, moisture and protein are presented for fresh beef in Table 1, fresh pork in Table 2, and cooked sausages in Table 3.

Statistical analyses of the difference between the two methods for the three types of products for fat, moisture and protein are presented in Tables 4, 5, and 6, respectively. Mean differences between results for all products combined indicated that the Hobart FMP-1 results were 0.38% higher for fat, 0.06% lower for moisture and 0.30% higher for protein. The mean differences for specific products indicated that there was less difference between the means for cooked sausage products than for fresh meats. However, this difference was attributed to two specific product types and proximate components. Specifically, significant (p <

0.05) differences in mean values between the methods of analysis were found for the fat level of fresh pork and the protein level of fresh beef. An examination of the standard deviations between the two methods indicated that these differences were not due to individual mean variations, as fat levels in pork and protein levels in beef showed mean and standard deviation differences of 0.96 ± 0.64%

and 0.62 ± 0.28%, respectively. These standard deviations were of similar magnitude to those found for the other components and types of meat products. Thus, the results suggest that a corrective factor (calibration) can be applied to the FMP-1 to adjust for these differences.

The application of the t-test and coefficient of variation statistics to the data indicated that differences be-

Table 1—Comparative duplicate determinations for percent fat, moisture and protein in seven fresh beef samples analyzed by the Hobart FMP-1 Analyzer and AOAC methods.

% Fat		% Moisture		% Protein	
Hobart FMP—1	AOAC	Hobart FMP—1	AOAC	Hobart FMP—1	AOAC
21.2, 20.6	19.49, 19.62	61.1, 61.5	62.52, 62.43	16.9, 17.1	16.54, 16.46
25.7, 25.9	25.22, 25.91	56.7, 56.7	56.86, 57.76	16.9, 16.7	16.20, 17.15
37.6, 37.3	38.17, 38.50	47.6, 47.4	46.31, 47.08	14.1, 14.7	13.74, 13.84
12.6, 12.0	12.46, 12.18	66.5, 67.0	67.23, 66.97	20.0, 20.1	19.17, 19.02
23.7, 23.1, 23.3	23.72, 24.22	57.8, 58.8, 58.2	57.73, 57.57	17.7, 17.3, 17.7	16.65, 16.74
41.7, 42.7, 42.3	42.39, 43.24	44.7, 43.7, 43.8	44.51, 44.56	13.0, 13.0, 13.3	12.46, 12.20
17.7, 17.3	17.67, 17.18	63.2, 64.0	64.10, 63.79	18.2, 17.9	17.32, 17.72

Table 2—Comparative duplicate determinations for percent fat, moisture and protein in six pork samples analyzed by the Hobart FMP-1 Analyzer and AOAC methods.

% Fat		% Moisture		% Protein	
Hobart FMP—1	AOAC	Hobart FMP—1	AOAC	Hobart FMP—1	AOAC
19.8, 19.7	18.77, 19.69	63.0, 63.1	63.12, 63.17	16.4, 16.4	15.96, 16.14
16.8, 16.4	14.92, 14.86	65.5, 65.7	65.99, 66.08	17.0, 17.0	18.02, 17.51
11.0, 11.6	10.62, 10.64	69.8, 69.4	69.86, 69.88	18.3, 18.2	17.15, 17.82
25.6, 25.2	24.27, 24.38	58.3, 58.7	59.13, 59.10	15.3, 15.4	14.68, 14.64
31.4, 31.6	31.42, 31.30	54.1, 53.9	52.92, 53.27	13.8, 13.8	14.23, 13.65
12.2, 12.7	10.81, 10.77	68.6, 68.2	69.02, 68.91	18.3, 18.2	18.30, 18.36

Table 3—Comparative duplicate determinations for percent fat, moisture, and protein in 12 cooked sausage product (bologna and frankfurters) samples analyzed by the Hobart FMP-1 Analyzer and AOAC methods

% Fat		% Moisture		% Protein	
Hobart FMP-1	AOAC	Hobart FMP-1	AOAC	Hobart FMP-1	AOAC
29.0, 29.0	28.97, 29.38	53.8, 53.8	52.93, 53.03	11.7, 11.7	10.93, 10.93
30.0, 29.7	29.93, 29.42	53.4, 53.7	53.01, 53.07	10.8, 10.7	10.16, 10.35
31.2, 31.0	29.82, 30.04	52.3, 52.4	52.32, 52.36	10.8, 10.8	10.49, 11.01
30.0, 29.5	30.26, 30.13	53.2, 53.6	53.44, 53.45	11.2, 11.2	10.67, 11.22
29.3, 28.4, 28.4	27.98, 28.42	53.6, 54.5, 54.5	54.71, 54.72	11.2, 11.2, 11.3	11.32, 10.98
30.0, 29.4	28.80, 28.52	53.3, 53.7	53.45, 53.44	10.9, 11.0	11.18, 10.52
30.1, 29.9	29.50, 29.14	52.8, 52.9	52.57, 52.48	11.4, 11.4	11.14, 11.18
29.5, 29.7	29.64, 29.74	53.4, 53.4	53.25, 53.23	11.0, 10.9	11.00, 11.08
30.1, 29.8	29.58, 29.34	53.3, 53.7	53.71, 53.65	10.4, 10.4	11.05, 10.91
29.0, 28.8	28.15, 28.23	54.4, 54.6	54.77, 54.82	10.9, 10.9	10.52, 10.25
28.9, 29.3	27.89, 28.32	54.7, 54.4	54.56, 54.68	10.3, 10.3	10.96, 10.08
28.9, 28.7	29.80, 29.68	53.8, 54.1	53.67, 53.64	11.5, 11.5	10.57, 10.76

Table 4—Statistical analysis of fat determinations by sample group of the results for the Hobart FMP-1 Analyzer and AOAC methods of analysis

Product	No. of samples	Means		Results between methods Hobart FPM-1 minus AOAC			Repeatability[a]		Comparison of results Hobart FPM-1 vs AOAC	
		Hobart	AOAC	Mean Dif.	Std Dev	Range	Hobart	AOAC	C.V.	t-values
Beef	7	25.65	25.71	−0.06	0.75	−0.89–1.34	0.411	0.386	2.92	−0.22
Pork	6	19.50	18.54	0.96	0.64	0.14–1.71	0.286	0.271	3.28	3.72[b]
Beef & Pork combined	13	22.81	22.40	0.41	0.85	−0.89–1.71	0.359	0.326	3.73	1.73
Cooked sausage products	12	29.54	29.19	0.35	0.65	−0.94–1.17	0.286	0.221	2.20	1.83
All products combined	25	26.04	25.66	0.38	0.75	−0.94–1.71	0.326	0.281	2.88	2.53[b]

[a] Std dev between duplicate determinations $(\sqrt{\Sigma d^2/2n})$ (Youden and Steiner, 1975).
[b] Significant at the 95% probability level.

Table 5—Statistical analysis of moisture determinations by sample group of the results for the Hobart FMP-1 Analyzer and AOAC methods of analysis

Product	No. of samples	Means		Results between methods Hobart FPM-1 minus AOAC			Repeatability[a]		Comparison of results Hobart FPM-1 vs AOAC	
		Hobart	AOAC	Mean Dif.	Std Dev	Range	Hobart	AOAC	C.V.	t-values
Beef	7	56.88	57.10	−0.22	0.70	−1.18−0.80	0.470	0.386	1.23	−0.83
Pork	6	63.19	63.37	−0.18	0.56	−0.62−0.90	0.218	0.110	0.89	−0.78
Beef & Pork combined	13	59.79	59.99	−0.20	0.61	−1.18−0.90	0.375	0.293	1.02	−1.18
Cooked sausage products	12	53.63	53.54	0.09	0.37	−0.52−0.82	0.226	0.043	0.69	0.89
All products combined	25	56.83	56.89	−0.06	0.52	−1.18−0.90	0.313	0.213	0.92	−0.57

[a] Std. dev between duplicate determinations ($\sqrt{\Sigma d^2/2n}$) (Youden and Steiner, 1975).

Table 6—Statistical analysis of protein determinations by sample group of the results for the Hobart FMP-1 Analyzer and AOAC methods of analysis

Product	No. of samples	Means		Results between methods Hobart FPM-1 minus AOAC			Repeatability[a]		Comparison of results Hobart FPM-1 vs AOAC	
		Hobart	AOAC	Mean Dif.	Std Dev	Range	Hobart	AOAC	C.V.	t-values
Beef	7	16.71	16.09	0.62	0.28	0.12−0.95	0.238	0.290	1.68	5.90[b]
Pork	6	16.51	16.37	0.14	0.58	−0.76−0.77	0.500	0.300	3.51	0.58
Beef & Pork combined	13	16.62	16.22	0.40	0.49	−0.76−0.95	0.178	0.295	2.95	2.92[b]
Cooked sausage products	12	10.98	10.80	0.18	0.41	−0.58−0.84	0.041	0.294	3.73	1.54
All products combined	25	13.91	13.62	0.30	0.46	−0.76−0.95	0.131	0.294	3.31	3.21[b]

[a] Std. dev between duplicate determinations ($\sqrt{\Sigma d^2/2n}$) (Youden and Steiner, 1975).
[b] Significant at the 95% probability level.

tween the two methods were significant ($p < 0.05$) for only the fat content of pork and the protein content of beef.

The statistical analyses indicated specific points which would require further adjustments and analyses to provide improvement in agreement of the Hobart FMP-1 with AOAC methods. It is obvious that isolated, specific product types and components produced the major differences indicated by the statistical analysis of the data. The Hobart FMP-1 analyzer can be calibrated by the manufacturer for specific product types to reduce mean differences and eliminate the biases which result for various components. It was concluded that the instrument would be acceptable for practical meat processing operations with the appropriate adjustments.

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Stability and Gel Strength of Frankfurter Batters Made with Reduced NaCl

R. C. WHITING

─────────────── ABSTRACT ───────────────

A detailed examination of fat emulsification and water-binding capacities in frankfurter batters and the cooked batters' textural responses to lowered levels of added sodium chloride was conducted. In response surface analyses of water and fat exudations and gel strength with varying compositions of fat, water, meat, and salt, the area of maximum stability decreased and moved toward a higher fat content when the salt was reduced from 2.5 to 1.5%. Gel strength increased with increasing content of lean meat and decreased with lowered salt levels. When salt, pH, chopping temperature, and cooking temperature were varied from the standard conditions, water exudate was generally affected first and most intensely while the gel strength was second. Fat release did not occur until more extreme conditions were encountered.

INTRODUCTION

EPIDEMIOLOGICAL, animal, and human studies strongly suggest that the consumption of sodium by some Americans should be curtailed to reduce the development of high blood pressure and subsequent cardiovascular diseases (Sebranek et al., 1983). Meat products significantly contribute to this dietary sodium. Of the average daily consumption of 10-12g NaCl, approximately 3-4g are added during food manufacture, with about 1g of this from meat products.

The salt concentration affects flavor, microbial shelf life and safety, and texture of meat products. Studies examining various ways to reduce the sodium content of meat products were reviewed recently by Maurer (1983) and Terrell (1983). The approaches generally involved (1) reducing the addition of NaCl, (2) substitution of other chloride salts for NaCl, (3) addition of other ingredients, and/or (4) alteration of processing techniques.

The emulsion characteristics of a frankfurter batter have received considerable emphasis (Webb, 1974; Acton et al., 1983). Salt solubilizes myofibrillar proteins which denature on the surface of the fat particles during comminution to form a stable emulsion. Recognizing the role of other proteins in the viscous colloidal solution that stabilizes the fat droplets, Swasdee et al. (1982) suggested the term "meat batter" to describe the system. More recently, the gelation aspects of the comminuted meat-salt brine-fat system have been studied (Lee et al., 1981). During thermal processing, the batter gels to produce the pseudoplastic solid characteristic of a frankfurter or bologna. This process is also a function of myofibrillar proteins (Acton et al., 1983).

For an effective reduction in the sodium content of frankfurters, the relationship between the meat batter's stability and texture as the sodium chloride content is reduced must be defined. In this report the compositional effects at different salt levels on the water binding ability, fat holding ability, and gel strength are presented. The characteristics of batters made with normal and reduced salt levels were determined under varying conditions of pH, chopping temperature, and cooking temperature.

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MATERIALS & METHODS

Meat

Fresh beef bottom rounds and pork adipose tissues were obtained from local abattoirs or wholesalers. The lean beef was trimmed of fat and gross connective tissue, ground coarsely, and stored at 1°C until used. Samples were analyzed for protein, fat, and moisture contents by the Kjeldahl, Soxhlet, and oven drying methods, respectively (AOAC, 1975). The beef lean and pork fat were further ground separately through a 3/16 in. plate prior to use.

Formulation and processing

The standard batter formulation of 200g contained 106g lean beef, 52g pork adipose tissue, 37g ice, and 5.0g NaCl. When the quantity of water was 40g or less, it was added as −29°C ice. All ingredients were added to the chopping bowl of a food processor (Cuisinart CFP-9) and chopped with brief interruptions to scrape sides of the bowl and measure the temperature until 16.0 ± 0.5°C was reached (Whiting and Miller, 1984).

Two 30 ± 0.1g aliquots of the batter were weighed into 50 mL glass centrifuge tubes (i.d. 2.5 cm) and centrifuged at 50 X g for 10 min. The centrifuge tubes were stoppered and placed into a 70-75°C water bath to cook for 30 min. Immediately after removal from the water bath, the water and fat exudates were decanted into calibrated conical centrifuge tubes for measurement (Morrison et al., 1971). Total exudate was the sum of the water and fat exudates.

The gel remaining in the centrifuge tube was allowed to cool to ambient temperature. Gel strength was determined by placing the tube vertically in a rack placed on the platen of an Instron Universal Testing Machine and forcing a 1/4 in. diameter, flat-bottomed rod through the gel at 500 mm/min. The maximum force of the initial penetration was recorded.

Statistical analyses

Water and fat releases and penetration forces of the two centrifuge tubes from a batter were averaged as one replicate. The effect of batter composition was analyzed by response surface methods (Henika, 1972; Hare, 1974). A second order mathematical model was used to fit the surface to the data.

RESULTS & DISCUSSION

THE STANDARD ADDITION of 2.5% NaCl is representative of the current industry practice for meat emulsion products (Terrell and Brown, 1981). The reduced sodium formulation contained 1.5% NaCl with the 1% reduction of salt compensated by an increased water content. The 2.5 and 1.5% NaCl formulations corresponded to 3.9 and 2.4% brine, respectively. The 1.5% salt level was picked as being an extreme reduction that would still possess frankfurter properties. Based on the proximate analyses, our uncooked standard batters averaged 12.0% protein, 24.3% fat, and 61.4% water. These values are intermediate between red meat and poultry frankfurters (USDA, 1980).

Composition

The composition of the batters was varied to develop response surfaces representing the stability of the batters. The response surfaces were calculated and plotted at both levels of salt by normalizing the lean meat, adipose tissue, and added water content. The three axes represent the percentages of the three major ingredients and sum to 100%

for each formulation. Lean meat varied from 10-90% of the formulation, adipose tissue from 5-60%, added water 5-40%, and salt 1.5-2.5%. Morrison et al. (1971) showed that a meat emulsion made with soybean oil had cook stability only within a limited range of water contents. The 2.5% salt batters (Fig. 1) corroborate their data; high total exudates were found at both low and high water contents. The 3 mL exudate/30g batter was the same criterion used by Morrison et al. (1971) for 90% stability. The response surface was not an ideal fit with 2.5% salt ($R^2 = 0.85$) because the area of minimum exudate was at a higher adipose tissue content than the contour lines indicate. Maximum stability was at 15-25% added water and 48% adipose tissue, which is neither a legal nor a desirable composition. When salt was reduced to 1.5% (Fig. 1), more extract was released from the batter upon cooking and no area on the response surface had less than 3.0 mL exudate ($R^2 = 0.94$) although one data point had 1.8 mL exudate. The standard frankfurter composition with 2.5% salt had 3.2 mL extract/30g; the only compositions made with 1.5% salt that equalled or surpassed this value had 48% adipose tissue.

The response surfaces for water exudate (Fig. 2) show minimal water loss with 15-20% added water and decreasing water loss with increasing fat content. When the salt was reduced the water extract increased over the entire response surface. The R^2 values for the 2.5 and 1.5% salt response surfaces were 0.93 and 0.99, respectively.

The data for fat exudate (Fig. 3) show little or no release until formulations had 48% added adipose tissue, except when added water was 5% of the batter. Even with 1.5% salt, fat release was relatively low at formulations around the standard composition, and the two response surface contours were similar. R^2 values were 0.77 and 0.83 for the 2.5 and 1.5% salt frankfurters, respectively.

The gel strengths (Fig. 4) measured by penetration force were most dependent upon the percentage of lean tissue. The 2.5% salt formulation had a slightly greater penetration force than the 1.5% formulation over the entire response surface. The surfaces were saddle shaped although only one slope fills the area of the frankfurter compositions. R^2 values were both 0.98.

Total chopping time was an approximation of the batter's viscosity and was also highly dependent upon composition. Fig. 5 shows combined data for the 1.5 and 2.5% salt levels ($R^2 = 0.98$); the salt level had relatively little effect on the time needed to reach 16°C. Using −29°C ice in the low water formulations had some effect in making the chopping times more uniform, but times still varied from 25-369 sec. Lowest times were for formulations that had only 5% added water. Times for the standard formulations averaged 84 sec. Hamm (see Acton et al., 1983) reported that viscosity was inversely proportional to the water content and directly proportional to protein and/or fat content.

Salt content

This experiment examined the effect of salt content alone in greater detail. Batters were made with varying salt additions; the added salt replaced an equal weight of water (ice) to keep the total brine constant. The release of water began to increase when the salt levels were less than 2.0% (Fig. 6). The release of fat, however, did not begin until the salt levels were less than 1.0%. The strength of gels did not show a sharp change at any salt level, and decreased
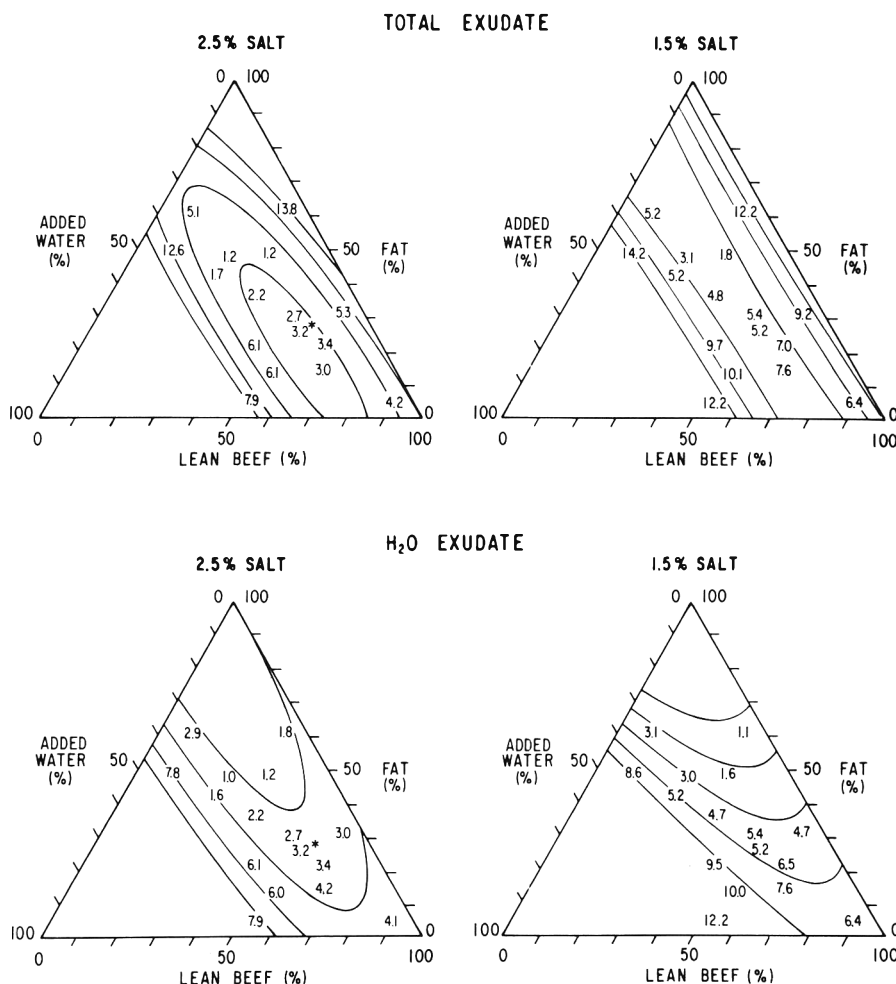
TOTAL EXUDATE

Fig. 1—Response surfaces of total exudates as affected by changes in composition and salt levels. Each number represents the mean of two batters each measured in duplicate. Units are milliliters total exudate per 30g of batter. Contour intervals are 12, 9, 6, and 3 (2.5% salt) mL exudate/30g. The standard composition is marked with an asterisk.

H₂O EXUDATE
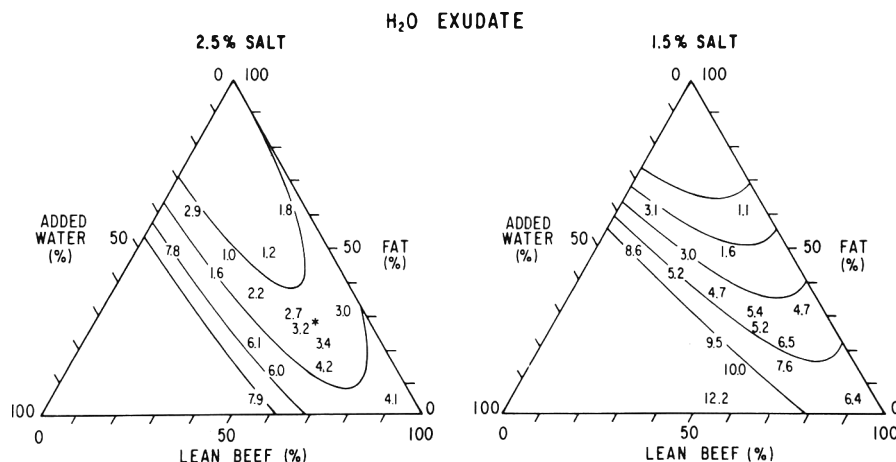
Fig. 2—Response surfaces of water exudates as affected by changes in composition and salt levels. Units are milliliters water exudate per 30g of batter. Contour intervals are 8, 6, 4, 2, and 0 (1.5% salt) mL exudate/30g. Symbols are the same as on Fig. 1.

Fig. 3—Response surfaces of fat exudates as affected by changes in composition and salt levels. Units are milliliters fat exudate per 30g of batter. Contour intervals are 6, 4, 2, and 0 mL exudate/30g. Symbols are the same as on Fig. 1.
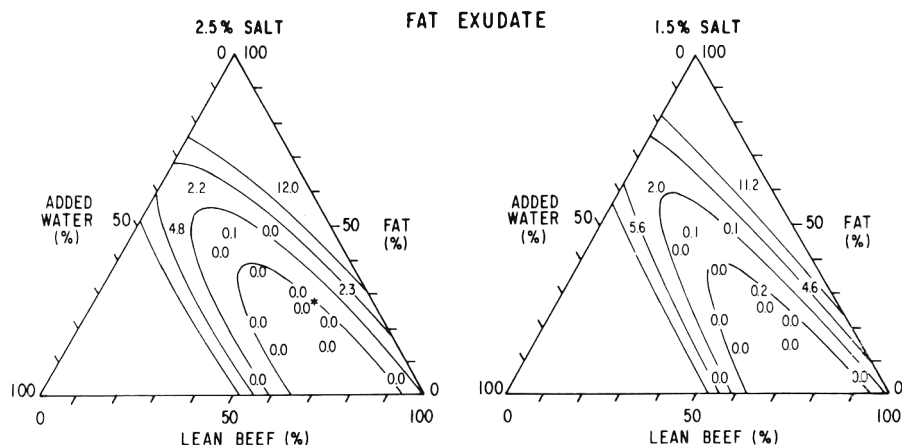
Fig. 4—Response surfaces of gel strengths as affected by changes in composition and salt levels. Units are grams (force). Contour intervals range from 0 – 1400g in steps of 200g. Symbols are the same as on Fig. 1.
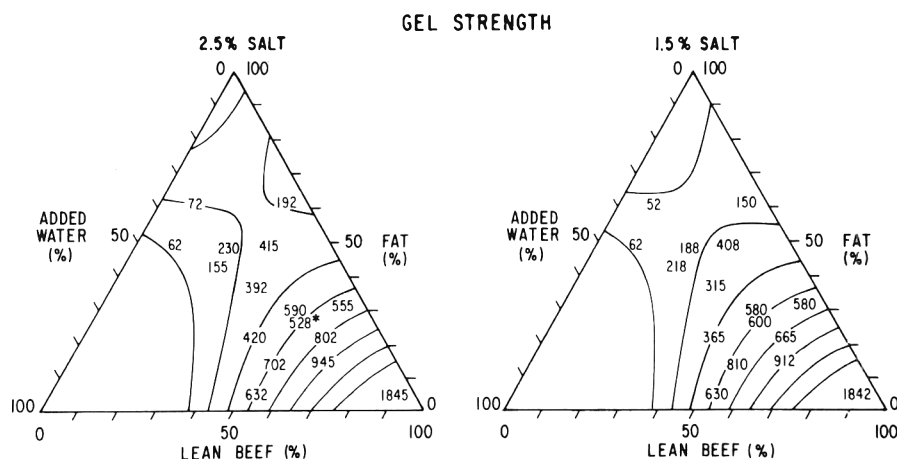
with decreasing salt levels over the entire 0.5-3.5% salt range tested. The penetration test had greater variation than the two stability tests in this and subsequent experiments.

These data were in agreement with other kinds of measurements involving salt and meat systems. Swift and Sulzbacher (1963) showed that the emulsifying capacity of a meat slurry increased with increasing salt concentration and this increase was accompanied by an increasing extraction of protein. Shults et al. (1972) showed that water losses from ground beef during cooking decreased as salt content increased. Ishioroshi et al. (1979) reported that although the solubility of purified myosin increases with salt, the gel strength was greatest with 0.2-0.3M salt, depending on the time between extraction and gel formation.

When salt levels were decreased, the water binding ability failed first. The gel strength declined as salt declined but did not abruptly fail at any salt concentration. Fat release did not begin until the water loss had greatly increased and the gel strength had declined about 45%.

pH

The pH of the batters was adjusted from 5.2 to 6.3 by additions of 1M HCl or NaOH after the first 10-15 sec of chopping. The maximum addition to the 200g of batter was 2.5 mL resulting in minimal change in batter composition. The pH was measured at the end of the chopping when the batter reached 16°C by direct insertion of pH electrodes with a temperature compensator.

Batters made with 1.5% salt were more susceptible to breakdown than those made with 2.5% salt (Fig. 7). Again, water exudate was more affected than fat exudate or gel strength. The 1.5% salt batters showed extensive water loss below pH 5.70, whereas the 2.5% salt batters failed at pH 5.55. Fat exudation was not important until the pH was
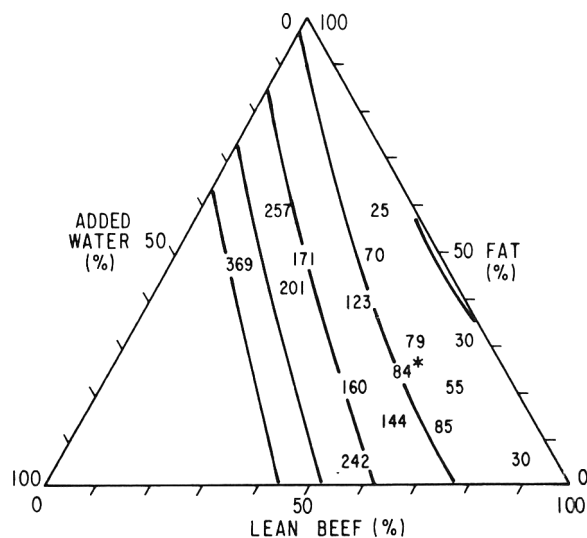
Fig. 5—Response surface of chopping times (sec) as affected by composition. The 1.5% and 2.5% salt levels are combined, each point represents the mean of four batters each measured in duplicate. The standard composition is marked with an asterisk. Contour intervals are 360, 270, 180, 90, and 0.

less than 5.4; however, the 1.5% salt batters tended to lose more fat than 2.5% batters. The penetration forces showed that reduced salt gels generally had less strength than the 2.5% gels. Maximum gel strength occurred at approximately pH 5.8; gel strengths declined slightly as the pH increased.

A limited amount of data exists on the pH effects in meat systems; however, the following reports of experi-
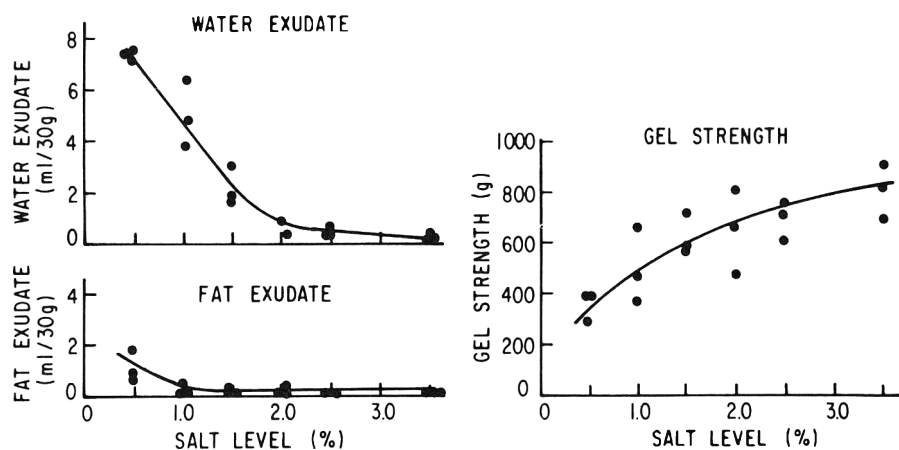
Fig. 6—Water exudates, fat exudates, and gel strengths of batters made with varying salt levels. Each point represents a single batter measured in duplicate.
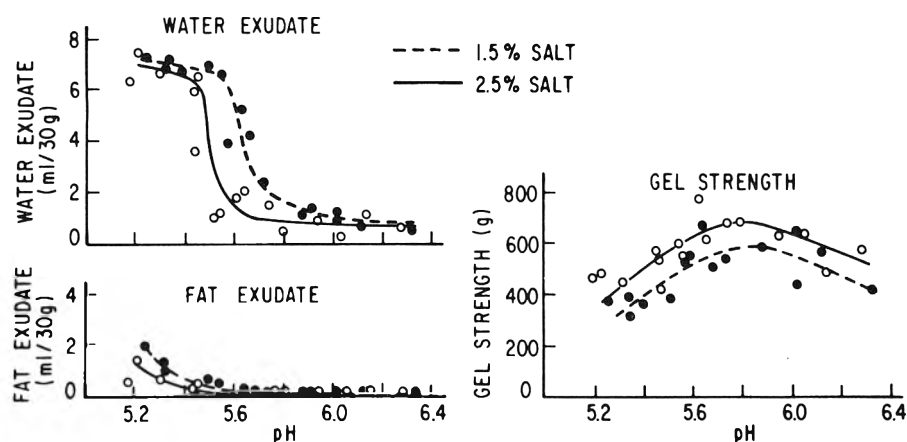
Fig. 7—Water extracts, fat extracts, and gel strengths of batters made with varying pH. Each point represents a single batter measured in duplicate.

ments with protein extracts indicated changing properties when at pH values less than 6. Acton et al. (1981) found natural actomyosin gels had a spongy texture at pH 5.0-5.5 and a uniform and opaque texture at pH 6.0 and above. However, the maximum gel strength occurred with the spongy texture. Gel strengths of purified myosin were maximum at pH 6.0 (Ishioroshi et al., 1979) and the minimum protein concentration for gelation of muscle extracts was at pH 5.8-6.1 (Trautman, 1966). The emulsifying capacity of muscle extracts was lower at pH 5.5 than 6.5 (Whiting and Richards, 1978); however, fat exudation was not apparent in the more concentrated meat batters used in this study.

Chopping temperature

Stability of the batters was greatly affected by chopping temperature as well as salt concentration (Fig. 8). For temperatures from 12-28°C the chopping times varied from 68-249 sec, respectively. Salt concentration did not affect chopping times except at the highest temperatures where 1.5% salt required slightly more time. The water exudate was greater at 12 and 16°C with 1.5% than 2.5% salt and began to increase at a lower temperature with 1.5% salt. The fat exudate likewise began to increase at a lower temperature in the reduced salt batters, 20°C vs 24°C. The penetration forces in the 2.5% salt batters declined slightly as the chopping temperature increased. The penetration forces in the 1.5% salt batters were always less than the 2.5% and declined at chopping temperatures above 20°C.

The temperature where the stability of the batter decreases was shown to be dependent on the characteristics of the fat by Townsend et al. (1968) who found that a phase change in pork fat begins at 18°C which coincided with a loss of emulsion stability. Lee et al. (1981) showed by elec-

tron micrographs that fat started to soften and the droplets started to coalesce at 21°C and that fat channels formed at 26°C. However, there is evidence that the proteins have a role in determining the temperature stability and would be expected to show a response to salt levels. The change in emulsion stability between 16 and 22°C was attributed by Jones and Mandigo (1982) to increasing water loss. Deng et al. (1981) ascribed the failure of water binding in a meat batter at 18°C to an increased protein-protein binding at the expense of protein-water interaction, and that fat separation would occur in 2.0% salt batters when chopping temperature exceeded 20°C. Lee et al. (1981) and Jones and Mandigo (1982) defined the comminuted meat batter as a gel-type emulsion where stability depends on the distribution of fat at the beginning of the gel matrix formation and the rigidity of the gel. The increasing stability from a thickening protein film around the fat globule as the chopping temperature increases is opposed by the film becoming too rigid and a loss of integrity of the protein-gel matrix.

The greater losses of water with reduced salt indicate the necessary role of salt for optimal gel formation. However, with 1.5% NaCl the proteins were able to completely bind the fat until temperatures above 20°C were reached.

Cook temperature

Batters of standard composition with either 1.5 or 2.5% salt were cooked for 30 min in water baths at 50, 60, or 70°C. No water or fat exudate occurred at 50°C, but gel strength was low (Fig. 9). Increasing the temperature to 60°C had little effect on water loss but cooking at 70°C increased water loss greatly. There were no fat losses at any temperature. Gel strength, however, steadily increased as the cook temperature increased; values at 70°C were approximately twice those at 50°C. The 1.5% salt batters

Fig. 8—Water extracts, fat extracts, and gel strengths of batters chopped to differing temperatures. Each point represents a single batter measured in duplicate.
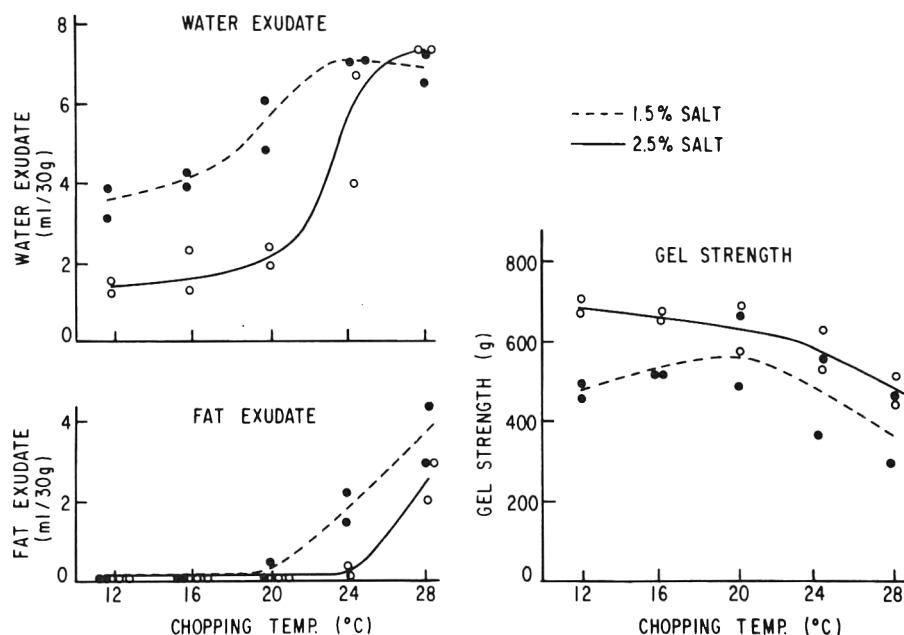
Fig. 9—Water extracts, fat extracts, and gel strengths of batters cooked for 30 min at varying temperatures. Each point represents the mean of four batters, each measured in duplicate.
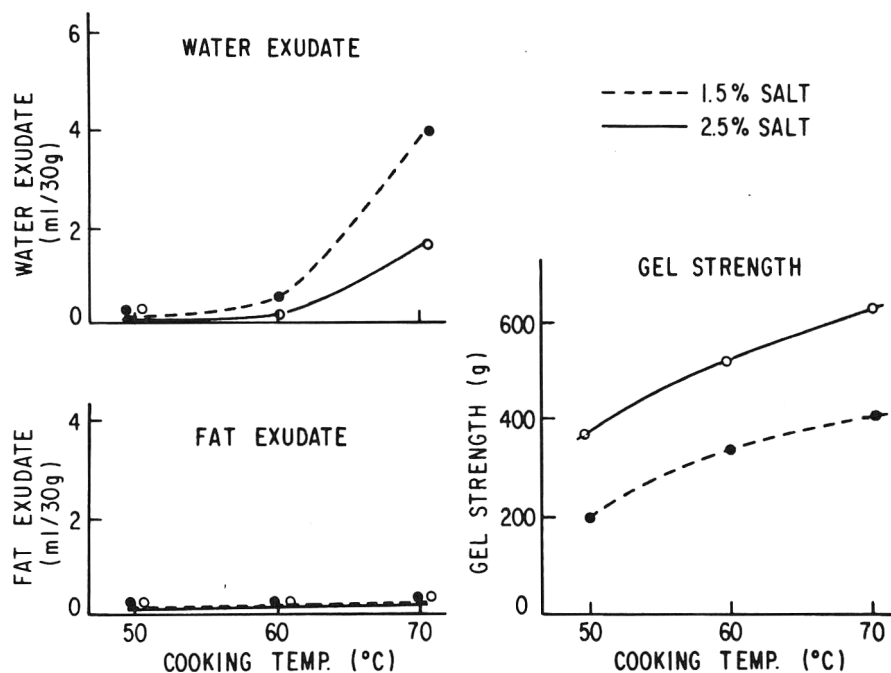
had greater water losses at 70°C and consistently lower gel strengths than the 2.5% salt batters.

The strength of the gels formed upon heating would primarily be a result of interactions between salt-soluble proteins (Acton et al., 1983). Ishioroshi et al. (1979) found myosin gels reached their maximum shear modulus at 60°C; however, Acton et al. (1981) observed that natural actomyosin gels increased in gel strength from 30-80°C. Siegel and Schmidt (1979) reported myosin binding ability steadily increased from 45-80°C. Lee et al. (1981) found heating of meat emulsions to 70°C reduced the retention of both fat and water relative to 60°C.

In summary, these results of batters with the normal 2.5% salt levels made with the food processor supported the various literature reports. This paper clearly showed the importance of salt in achieving the desirable characteristics and what changes occurred when the salt was reduced. In all experiments, the first attribute of the meat batter to fail with reduced salt alone or with another stress was the water

binding. The gel strength was second while the fat emulsification, the traditional point of reference in meat emulsions, was affected least.

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Addition of Phosphates, Proteins, and Gums to Reduced-Salt Frankfurter Batters

R. C. WHITING

————————ABSTRACT————————

Reducing the added salt levels in frankfurter batters from 2.5 to 1.5% decreased water binding abilities. Gel strength was less affected and fat release least affected. Tripolyphosphate and pyrophosphate were very effective at levels of 0.1% in restoring the water binding and gel strength. The effectiveness of these phosphates was nearly identical although they have opposing effects on batter pH. Soy isolate and rennet-treated, calcium-reduced dried skim milk improved the batters when added at 1 and 3%. Additions of alginate or especially xanthan gums (0.1–0.3%) improved the water binding but were very detrimental to the gel strength.

INTRODUCTION

SODIUM CHLORIDE REDUCTION in meat products is an important component in the goal of an overall decrease in dietary sodium and the potential lessening of the incidences of high blood pressure and subsequent cardiovascular diseases (Sebranek et al., 1983). A companion paper (Whiting, 1984) shows that reducing the added salt in a frankfurter batter caused an extensive loss of water binding ability. Gel strength was also reduced but fat exudation was not a problem unless salt reductions or processing variable changes were extreme. It is desirable to maintain the functional attributes of meat batters through processing alterations with concomitant sodium reduction.

Phosphates have been added to meat products to improve their binding and water-holding properties. Ellinger (1972), Knipe (1983), and Trout and Schmidt (1983) reviewed the uses of phosphates in meat products and have listed possible modes of action: (1) buffering (raising) the pH, (2) increasing the anionic electrolytes, (3) sequestering cations, (4) raising the ionic strength, and (5) dissociating actomyosin. Phosphates may also crosslink proteins or block reactive sites. Hargett et al. (1980) reported that sodium acid pyrophosphate (SAPP) was the most effective phosphate for improving the firmness and springiness of frankfurters.

Proteins and other extenders have also been used in frankfurters to improve their textural properties (Terrell, 1978). Gums are also capable of binding water and forming gels (Klose and Glicksman, 1972). In this study representatives of these additives were evaluated for their capabilities to affect fat and water exudation and gel strength in frankfurter batters made with a 40% reduction in added salt.

MATERIALS & METHODS

FRANKFURTER BATTERS were made from fresh beef bottom rounds and pork adipose tissues as described previously (Whiting, 1984). Phosphates added in concentrations up to 0.25% were sodium mono- and dibasic phosphate (Pi), sodium acid pyrophosphate (Ventron Inc.), and sodium tripolyphosphate (Stauffer Chemical Corp.). Xanthan gum (Meer Corp.) and sodium alginate (Kimits Corp.) were also tested at 0.1 and 0.3%. Protein extenders used at 1.0 and 3.0% were soy isolate (Supro 620, Ralston Purina Co.), gluten (Supergluten-80, Industrial Grain Prod.), rennet-treated, calcium-reduced, dried skim milk plus calcium lactate (Alatek,

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Alaco Co.), and collagen prepared as described by Whitmore et al. (1972).

Evaluations of water binding, fat emulsification, and gel strength were performed in duplicate as previously described (Whiting, 1984). The interaction of salt content, pH, and SAPP level was analyzed by factorial design analyses of variance (SAS, 1979).

RESULTS & DISCUSSION

Phosphate addition

Preliminary trials showed that addition of 0.25% SAPP to the batters reduced their pH from 5.8 to 5.5. Therefore, a factorial experiment was designed with 2.5 and 1.5% salt batters, SAPP, and adjustment of the pH to pH 5.5 or 5.8 with HCl or NaOH independently of SAPP (Table 1). Water exudate was greater at pH 5.5 than 5.8. This was especially significant when 1.5% salt was used. Addition of SAPP greatly reduced the water exudate such that 1.5% salt emulsions with 0.25% SAPP and pH 5.5 were nearly equal to the 2.5% salt batters with no phosphate or pH adjustment. Analysis of variance indicated all three main effects (salt, pH, SAPP) were highly significant ($p < 0.01$); all the two-way interactions were highly significant ($p < 0.01$); and the three-way interaction was significant ($p < 0.05$).

No fat exudate was observed from the 2.5% salt batters without phosphate or pH adjustment and exudate from the 1.5% salt batter was negligible. Lowering pH to 5.5 did not cause any fat exudate with 2.5% salt but resulted in exudate with 1.5% salt which was prevented by addition of 0.12% pyrophosphate. Analysis of variance for fat exudate showed this effect to be highly significant ($p < 0.01$).

Gel strength data were less consistent. Reducing salt concentrations decreased the penetration forces, except for an anomalous value in the batter with 1.5% salt, pH 5.5, and 0.25% SAPP. Lowering of pH generally reduced the gel's strength, and adding pyrophosphate increased the penetration force. Analysis of variance indicated only salt level ($p < 0.05$) and SAPP level ($p < 0.01$) as significant. Hargett et al. (1980) found that SAPP improved texture of frankfurters only slightly, but did not improve moisture retention or cook yields. These frankfurters, however, contained 3.0% added salt. SAPP, therefore, appears to be more effective when the batter is of marginal stability.

Table 1—Water exudate, fat exudate, and gel strength of batters made with sodium acid pyrophosphate and controlled pH

| | SAPP (%) | 1.5% Salt | | 2.5% Salt | |
| | | pH | | pH | |
		5.5	5.8	5.5	5.8
Water exudate (mL/30g)	0.0	6.8	2.0	1.7	0.7
	0.12	1.9	0.4	0.2	0.0
	0.25	1.0	0.4	0.1	0.0
Fat exudate (mL/30g)	0.0	1.1	0.1	0.0	0.0
	0.12	0.0	0.0	0.0	0.0
	0.25	0.0	0.0	0.0	0.0
Gel strength (g)	0.0	390	612	665	640
	0.12	565	700	788	835
	0.25	995	768	845	872
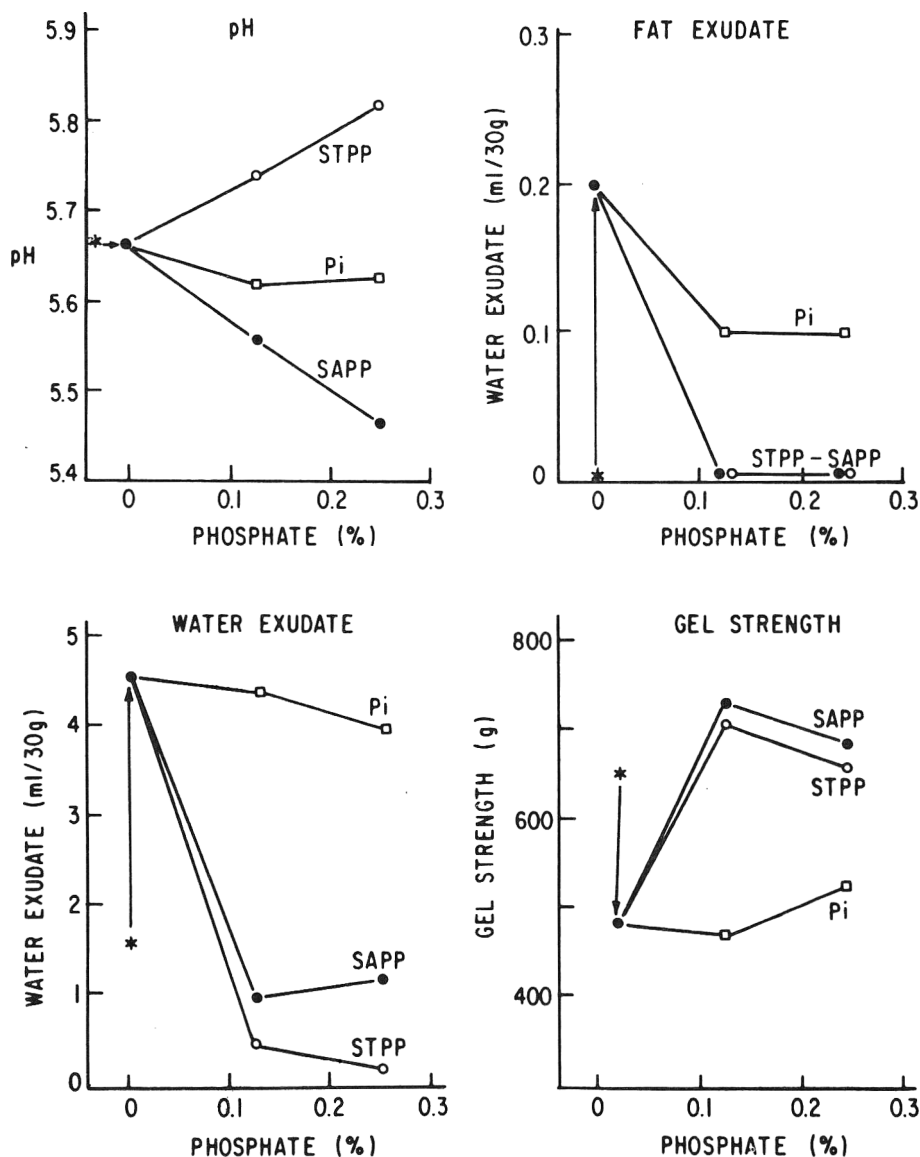
Fig. 1—pH values, water exudates, fat exudates, and gel strengths of reduced-salt batters with added phosphates. The 2.5% salt control is marked with an asterisk and the arrow indicates the change in the 1.5% control. Phosphate containing batters all had 1.5% salt. Values are means of two batters measured in duplicate. Orthophosphate (Pi), □; sodium acid pyrophosphate (SAPP), ●; sodium tripolyphosphate (STPP), ○.

Shults et al. (1972) demonstrated differing abilities of various phosphates to reduce shrink and increase water retention in intact beef. These investigators reported tripolyphosphate to be better than pyrophosphate and much better than orthophosphate. Fig. 1 shows the effect of phosphate type in improving the batter's properties when the salt was reduced. The arrow shows the change from 2.5% salt (*) to 1.5% salt with no added phosphate. The phosphates were added to batters containing 1.5% salt. The pH values were not controlled in this experiment; 0.25% SAPP reduced the pH from 5.66 to 5.46, 0.25% sodium tripolyphosphate (STPP) increased the pH to 5.82, while the sodium mono- and dibasic orthophosphates (Pi) were combined to have no effect on pH.

Water exudate increased from 1.6 to 4.3 ml when the salt level was reduced. Addition of STPP or SAPP to the 1.5% salt batters reduced the water exudate to values less than the 2.5% salt batters. Orthophosphate had no effect. The results generally confirm those of Shults et al. (1972). No fat exudate appeared when STPP or SAPP were added and exudation was reduced with Pi. Gel strength was completely restored with 0.12% STPP or SAPP, but was unaffected by Pi.

The average chopping times decreased with addition of 0.25% SAPP from 91 sec to 85.5 sec indicating an increase of the batters' viscosity. Addition of STPP had a lesser effect. However, Hargett et al. (1980) observed a lowered viscosity in emulsions with added SAPP.

Addition of binders

To determine whether other binders would potentially improve the properties of the meat batter, especially water binding, xanthan and alginate gums were added at 0.1 and 0.3% (Klose and Glicksman, 1972). Protein extenders used at 1.0 and 3.0% were soy isolate; gluten; rennet-treated, calcium-reduced, dried skim milk plus calcium lactate; and collagen. For comparison, batters were also made with 0.05% and 0.10% SAPP. All additives were included at the beginning of the chop as dry powders and replaced an equal weight of lean beef in the formulation. All batters except for the 2.5% salt controls had 1.5% salt.

Data (Fig. 2) from several experimental series were calculated as percentages of their respective 2.5% salt batters. Total exudate is shown since only small amounts of fat were released by a few formulations. The two gums reduced the water exudate to levels below the 1.5% salt controls, xanthan reduced water exudate below the 2.5% controls. However, the gel strength of these gels was very poor, especially with xanthan gum, agreeing with previous data on frankfurters containing xanthan gum (Fox et al., 1983). Soy isolate and rennet-treated, calcium-reduced,

Fig. 2—Total exudates and gel strengths of batters with added gums, proteins, or pyrophosphate. Except for the 2.5% salt control, all batters have 1.5% salt. Values were calculated as a percentage of their respective 2.5% salt controls. Standard deviations are indicated except for the 0.05% SAPP which was a single batter.
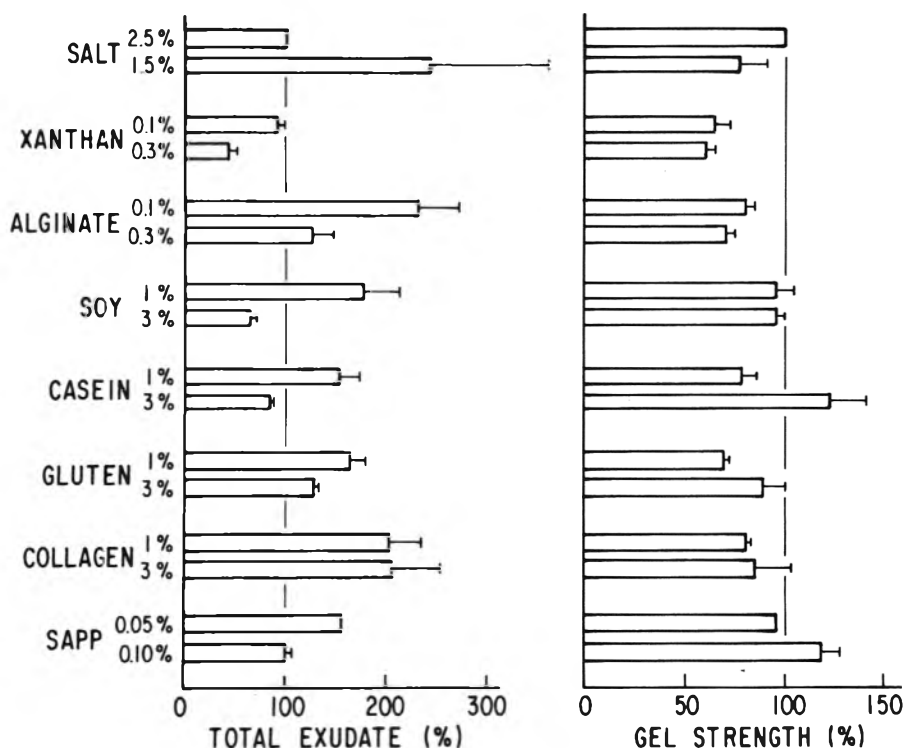
dried skim milk were both capable of reducing water extract and restoring gel strength. Gluten was less successful and collagen was poor at improving properties of low salt batters. As shown above, SAPP was effective even when added in amounts as low as 0.05%.

In previous studies, extenders generally have been found to be inferior to myosin for functionality in meat products (Smith et al., 1973; Lauck, 1975; Siegel et al., 1979; Rao et al., 1981; Fox et al., 1983). However, these extenders were generally added to good meat batters where the salt-soluble proteins provided ample water binding and gel strength. This work suggested that in less optimal systems, such as with reduced salt, beneficial effects may result from addition of one or combinations of these extenders. Addition of phosphate was very promising although further work is necessary to determine the mechanisms involved and the best form of phosphate to add. This brief survey of protein extenders also showed several had advantageous properties, additional study would undoubtedly find others and could also examine the sensory consequences of using extenders in reduced salt meat products.

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Emulsion and Storage Stabilities of Emulsions Incorporating Mechanically Deboned Poultry Meat and Various Soy Flours

L. D. THOMPSON, D. M. JANKY, and A. S. ARAFA

―――――――――― ABSTRACT ――――――――――

The stability of emulsions formulated with mechanically deboned poultry in combination with various levels (10%, 15%, 20%, and 25%) of rehydrated vegetable protein flour (VPF), 50% protein; soy concentrate flour (SCF), 70% protein; or soy isolate flour (SIF), 90% protein were determined. VPF emulsions had significantly better emulsion stability than those with SCF or SIF. Flour type had no effect on storage stability (2-thiobarbituric acid values, tensile strength, dominant wavelength, or sensory evaluation); however, SIF emulsions had significantly higher microbiological counts than emulsions incorporating VPF or SCF. Rehydrated flour level had no effect on stability with the exception of higher tensile strength values at lower rehydrated flour levels.

INTRODUCTION

MECHANICALLY DEBONED poultry meat is an inexpensive meat source which, because of its pasty consistency and lack of fibrous structure, has been predominantly used in emulsified products. Generally, mechanically deboned poultry meat has a high fat content when compared to hand deboned poultry meat, and it has been proposed that various types of soy products could be added to improve the functional and nutritional properties of the meat.

Soy products that are most frequently used in comminuted meat products include soy flours, grits, concentrates, and isolates. The end products remaining after the oil extraction of dehulled soybeans are ground to obtain varying particle sizes or further processed to remove nonprotein material. Soy flours and grits, both 40-60% protein, do not undergo further processing. They vary only in their particle size with soy flours passing through 100-mesh or smaller screen size, while soy grits are passed through a screen size ranging from No. 80-10.

To obtain concentrates and isolates, the product remaining after oil extraction undergoes further processing to remove most of the oil and water soluble nonprotein material. The National Soybean Processors Association defines soy concentrate as a product containing not less than 70% protein, while soy isolate contains not less than 90% protein (Smith and Circle, 1972).

Governmental efforts have been made to encourage the use of soy in emulsified meat products because of its low cost-to-protein ratio. One such effort involves the school lunch program developed by the USDA. The guidelines allow up to 30% substitution of rehydrated textured vegetable protein or rehydrated granular soy concentrate in meats used in the school lunch program (Rakosy, 1975). Mechanically deboned poultry meat could possibly be used, but there has been little research on the stability of emulsion-type products formulated with mechanically deboned poultry meat and soy products.

Cunningham (1977) substituted flaked texturized vegetable protein (FTVP) for ground Leghorn meat at 10%, 20%, and 30% levels. The 2-thiobarbituric acid values were consistently lower in the soy-substituted meat patties than

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the all-meat patties during six months frozen storage. Taste panelists rated patties with higher FTVP levels less tender and less desirable for flavor when thawed and microwaved. FTVP level had no effect on flavor scores when patties were pan-fried from either the frozen or thawed state.

Lyon et al. (1981) added isolated soy protein at 0% and 2% levels to rolls made with hand deboned poultry meat, and 0%, 10%, or 20% mechanically deboned poultry meat. Isolated soy protein increased cooked roll yields, but did not affect Hunter color values, subjective color scores, or textural attributes including springiness, hardness, cohesiveness, or chewiness.

Cunningham and Bowers (1977) combined ground White Leghorn meat with 10% textured soy protein (TSP); the soy patties had lower 2-thiobarbituric acid values and lighter more yellow color during refrigerated storage than did 100% chicken patties. Total aerobic plate counts, coliform counts, and yeast and mold counts were similar for fresh all-meat patties and 10% soy patties. Craven and Mercuri (1977), however, reported higher total aerobic plate counts after 10 days refrigerated storage in both beef and chicken patties containing texturized soy protein than patties with no soy. The authors concluded that meat extended with soy products may spoil sooner than all-meat products, possibly because of some stimulating effect of the soy additive on the microflora.

Nonextended ground beef had significantly lower psychrotrophic and mesophilic bacterial counts than beef extended with either 5%, 10%, 20%, or 40% texturized soy protein and stored 6, 8, and 10 days at 0°C (Draughon et al., 1982). Higher texturized soy protein level resulted in higher moisture and fat content. The authors suggested that increased microbial growth rates were related to increased water activity, since inclusion of soy at the higher levels resulted in higher moisture content.

The purpose of this study was to examine both the emulsion stability and storage stability of emulsions consisting of mechanically deboned poultry meat and one of three types of rehydrated soy flours (vegetable protein flour, soy concentrate flour, and soy isolate flour) at varying levels (10%, 15%, 20%, and 25%) when stored at 2°C for up to 8 wk.

MATERIALS & METHODS

Emulsion preparation

Frozen mechanically deboned poultry meat was obtained from a commercial processing plant, stored at −25 ± 2°C, and used within 3 wk. Prior to utilization, percent protein (12.51%), ether extract (23.22%), and moisture (63.29%) were determined according to AOAC (1970) methods while ash (0.98%) was determined by difference. The degree of initial fat oxidation in the raw meat (0.21 mg malonaldehyde/kg) was determined by the 2-thiobarbituric acid analysis of Tarladgis et al. (1960).

Three groups of emulsions were prepared, each consisting of mechanically deboned poultry meat combined with one of three types of rehydrated soy flour: vegetable protein flour (VPF), 50% protein; soy concentrate flour (SCF), 70% protein; or soy isolate flour (SIF), 90% protein. The rehydrated flours were added at 10%, 15%, 20%, or 25% of the emulsion formulation, for a total of 12

treatments. Rehydration levels for the flours were 1:2 (flour:water) for VPF, 1:3 for SCF, and 1:4 for SIF. These levels were selected to maintain a constant added protein concentration for each rehydrated flour level across the three flour types. In addition, an all meat reference emulsion was prepared to provide baseline data for emulsion stability of the particular source of mechanically deboned poultry meat used. The emulsion formulation included 96.8% mechanically deboned poultry meat and rehydrated soy flour; 0.50% seasonings (Griffiths #012-0940); 1.9% salt; 0.50% sugar; and 0.30% tripolyphosphates.

Each 2.7 kg batch was prepared by chopping frozen, mechanically deboned poultry meat in a Hobart silent cutter. A premeasured packet of salt, seasonings, sugar, and tripolyphosphates was added when a pasty consistency was obtained followed immediately by the addition of the rehydrated soy flour. The mixture was chopped to an end-point temperature of 13°C, then stuffed into Tee Pac® casings (6 cm in diameter) using a Voget® hand-cranked stuffer to make five 0.5 kg sausages per treatment. Sausages were held overnight at 2 ± 1°C, placed in a pan, and cooked in a smokehouse without smoke at 93.3°C for 1.5 hr. The temperature was raised to 121°C and cooking continued to an internal temperature of 71°C.

Emulsion stability

Samples from each raw emulsion were stuffed into three tared 60 ml polystyrene syringe barrels to determine emulsion stability by the method of Townsend et al. (1968). The syringe barrels were stoppered and held overnight at 2°C for percent cookloss, mL gel, mL fat, and released solids determinations.

Storage stability

Initially, and at 2, 4, 6, and 8 wk of storage (2°C), one cooked sausage (in casing) was selected from each treatment and analyzed for tensile strenth, 2-thiobarbituric acid value, microbiology, color, and sensory evaluation.

Tensile strength

Five slices (45 x 19 x 5 mm) from each sausage were obtained using an Oster® "Choice-Kut" meat slicer and a template. After 2 hr refrigeration (7°C), each sample was sheared at the center on a device developed by Swift and Ellis (1957) using a 2.5 x 1 cm bar. The tensile strength of each sausage was recorded as the mean value of the grams-force required to shear each of the five samples.

2-thiobarbituric acid values

The 2-thiobarbituric acid values were determined, in duplicate, by the method of Tarladgis et al. (1960) using a Beckman spectrophotometer at 538 nm and a 0.5 mm slit width.

Microbiological analysis

The microbiological sample was prepared from each cooked sausage by homogenizing 10g of sample and 90 mL sterile phosphate buffer in a sterile blender jar. Serial dilutions were prepared by transfering the appropriate 10 mL aliquot to 90 mL sterile phosphate buffer. Aerobic plate counts were made by inoculating duplicate prepoured plates of plate count agar (DIFCO), using aseptic technique, with 0.1 mL inoculum from the serial dilutions. A sterile bent-glass rod was used to distribute the inoculum evenly over the medium surface. Three sets of plates, including all dilutions, were prepared from each sausage with one set incubated at each of 7°C for 10 days, 20°C for 5 days, and 35°C for 48 hr. Aerobic plate counts at 7°C for 10 days and 35°C for 48 hr were also made from the reference all-meat raw and cooked emulsion. Coliform counts were made, in duplicate, using the pour plate method (APHA, 1976), Violet Red Bile (VRB) agar (DIFCO), and 1 mL of inoculum from the serial dilutions previously described. The plates containing the VRB media (solidified) were overlaid with approximately 5 mL of VRB agar, incubated at 35°C for 24 hr, and typical coliform colonies enumerated.

Sensory evaluation

Taste panels were conducted using untrained panelists consisting of available faculty, staff, and students in the Poultry Science Department. Each tasting session utilized eight to ten panelists from this group with no attempt to control individual panelist participation. At the end of each storage period (0, 2, 4, 6, and 8 wk), one tasting session was conducted with emulsions from each soy flour

type (three sessions). In each tasting session, panelists were presented with four random-number identified emulsion samples; one from each rehydrated flour level within a flour type. Samples consisted of 1 x 4-5 cm cores from the sausages and were held at 7°C for 0.5 hr prior to serving. Panelists were asked to rate each of the four samples for flavor (0 = very undesirable, 10 = very desirable), juiciness (0 = very dry, 10 = very juicy), texture (0 = very tough, 10 = very tender), and acceptability (0 = very unacceptable, 10 = very acceptable) by marking their decision on an unstructured 10 cm line anchored at the extremities. Marks were converted to digital values by measuring the distance (cm) from the left anchor point to the evaluator's mark.

This arrangement provided for evaluation of rehydrated flour level within each flour type and storage time. Because of the random effects of time (storage) and panel (panelist identity), statistical comparison of sensory differences between flour types or storage times were invalid.

Color evaluation

The sausages were evaluated for color using a MacBeth MC-1010® reflectance colorimeter. Raw data were converted to values for dominant wavelength (DWL), excitation purity (EP), and luminosity (LUM) using the computer program developed by Fry and Damron (1971) with modifications for the MC-1010® colorimeter. An average value for DWL, EP, and LUM was calculated for each sausage by averaging triplicate evaluations from three cross sections of each sausage.

Statistical analysis

The entire experiment was conducted twice (trials) at two completely different times. Data were subjected to analysis of variance and Duncan's procedure as outlined by the Statistical Analysis Systems Institute, Inc. (1982). Since there was no significant trial x treatment interaction, data from the two trials were pooled.

RESULTS & DISCUSSION

Emulsion stability

The all-meat reference emulsion released similar amounts of fat and solids, but numerically higher amounts of gel and had a higher cookloss than the soy-added emulsions (Table 1). This would indicate that the addition of soy would improve the stability of mechanically deboned meat emulsions as previously observed by Lyon et al. (1981).

The emulsions formulated with VPF had significantly ($P < 0.05$) better emulsion stability than emulsions formulated with SCF or SIF, as exhibited by significantly lower cookloss, ml fat, and ml gel released (Table 1). Because the amount of protein added from each rehydrated flour type at a given level was equal, the amount of water added with each rehydrated flour type was different (VPF = 60% water, SCF = 75%, and SIF = 90%). Thus, there was more water to be lost in the emulsions made with SCF or SIF. According to Schut (1976), soy proteins probably do not act as emulsifying agents in meat systems,

Table 1—Stability of emulsions prepared with vegetable protein flour (VPF), soy concentrate flour (SCF), soy isolate flour (SIF), and no soy flour (control)[a]

Soy flour type	% Cookloss[b]	Released/100g emulsion		% solids released[e]
		mL fat[c]	mL gel[d]	
VPF	9.66[f]	0.59[f]	3.08[f]	0.23[f]
SCF	13.27[g]	1.49[g]	5.49[g]	0.21[f]
SIF	13.71[g]	1.85[g]	5.83[g]	0.15[f]
Reference	15.60	1.78	6.07	0.14

[a] Means within a column having different superscripts are significantly different (P < 0.05), n=40.
[b] % cookloss = (sample wt — cooked emulsion wt) X 100/sample wt.
[c] mL fat/100g emulsion = ml fat released X 100/sample wt.
[d] mL gel/100g emulsion = ml gel released X 100/sample wt.
[e] wt solids released X 100/sample wt.

but they do fortify the matrix of meat emulsions by immobilizing water within a stable gel that forms upon heating. The increased fat released from the SCF and SIF emulsions when compared to the VPF emulsions (Table 1) could also be related to the larger amounts of water in the rehydrated SCF and SIF. If large amounts of water are present in the emulsion, water retention and gel formation could be impaired, thus adversely affecting the ability of the emulsion to immobilize fat by entrapment within the gel (Schut, 1976).

Rehydrated flour level had no significant ($P > 0.05$) effect on percent cookloss or percent solids (Table 2); however, there was a numerical trend to decrease these components as rehydrated flour level was increased. Emulsions prepared with less than 20% rehydrated flour released significantly ($P < 0.05$) more fat and gel than emulsions prepared with rehydrated flour levels of 20 or more percent. The apparent improvement in emulsion stability with higher levels of rehydrated soy flour could be related to higher concentrations of protein available for the gelation phenomena described by Schut (1976).

Storage stability

Flour type had no significant effect on tensile strength (Table 3); however, emulsions prepared with 25% rehydrated flour had significantly ($P < 0.05$) lower tensile strength than that observed for emulsions made with either 10% or 15% rehydrated flour.

As storage time progressed from 0 to 8 wk, there was a significant ($P < 0.05$) increase in tensile strength (Table 3). It was concluded that this increase could have been caused by dehydration of the cooked sausages during storage.

Neither flour level nor type had a significant ($P > 0.05$) effect on rancidity as expressed by 2-thiobarbituric acid values (Table 3); however, as expected, there was a direct correlation between increased storage times and significantly ($P < 0.05$) increased rancidity. Flour type would not be expected to alter oxidation rate since all the flours tested were low in fats [1.0-0.1% (Horan, 1974)]. Flour level, however, could conceivably alter storage stability because as the level of flour was increased, there was a proportional decrease in mechanically deboned poultry meat, thus fat, content. This response was not observed in this study (Table 3).

The addition of rehydrated SIF to emulsions produced significantly ($P < 0.05$) higher aerobic plate counts at 20°C and 35°C in the cooked emulsions than did the incorporation of the other two flours (Table 4). Incorporation of SIF also produced significantly ($P < 0.05$) higher counts at 7°C than those observed for emulsions continaing VPF. Rehydrated flour level had no significant ($P > 0.05$) effect on aerobic plate counts at any of the incubation tempera-

tures tested (Table 4). The data from this experiment suggested that SIF had a higher initial microbial load of viable thermophilic organisms than VPF or SCF. This would not be surprising since the production of SIF would involve more extensive processing and heating that could provide increased opportunity for contamination and enhance selection for thermophilic organisms.

Alternatively, the higher microbial level in cooked

Table 3—Tensile strength and 2-thiobarbituric acid values (TBA) of emulsions prepared with vegetable protein flour (VPF), soy concentrate flour (SCF), or soy isolate flour (SIF)[a]

	Tensile strength (grams force)	TBA (mg malonaldehyde/1000g)
Flour type[b]		
VPF	267.21[e]	1.78[e]
SCF	248.00[e]	2.17[e]
SIF	265.94[e]	2.23[e]
Rehydrated flour level (%)[c]		
10	274.08[f]	2.30[e]
15	280.67[f]	1.94[e]
20	258.57[ef]	2.23[e]
25	228.58[e]	1.78[e]
Storage time (wk)[d]		
Initial	232.98[e]	0.78[e]
2	260.69[f]	1.62[f]
4	251.24[ef]	2.03[fg]
6	270.55[fg]	2.63[gh]
8	287.41[g]	3.26[h]

[a] Means within a column and a section having different superscripts are significantly different ($P < 0.05$).
[b] n = 40.
[c] n = 30.
[d] n = 24.

Table 4—Log counts of colony forming units (CFU) for aerobic plates (APC) incubated at 7°, 20°, and 35°C (for 10 days, 5 days, and 48 hr, respectively) for emulsions prepared with vegetable protein flour (VPF), soy concentrate flour (SCF), or soy isolate flour (SIF)[a]

	Log counts of CFU		
	APC 7°C	APC 20°C	APC 35°C
Flour type[b]			
VPF	3.77[e]	4.01[e]	3.34[e]
SCF	4.24[ef]	3.94[e]	3.38[e]
SIF	4.42[f]	4.70[f]	4.61[f]
Rehydrated flour level (%)[c]			
10	4.00[e]	4.23[e]	3.57[e]
15	4.13[e]	4.13[e]	3.77[e]
20	4.15[e]	4.25[e]	3.92[e]
25	4.29[e]	4.24[e]	3.85[e]
Storage time (wk)[d]			
Initial	<2.00[e]	2.23[e]	2.43[e]
2	4.44[f]	4.42[f]	4.06[f]
4	4.95[f]	4.72[fg]	4.10[f]
6	5.17[f]	4.62[fg]	4.10[f]
8	5.20[f]	5.16[g]	4.21[f]

[a] Means within a column and a section having different superscripts are significantly different ($P < 0.05$).
[b] n = 40.
[c] n = 30.
[d] n = 24.

Table 2—Stability of emulsions prepared with one of four rehydrated soy flour levels (10, 15, 20, or 25%)[a]

Rehydrated soy flour level (%)	% Cookloss[b]	Released/100 g emulsion		% solids released[e]
		mL fat[c]	mL gel[d]	
10	13.05[f]	1.88[g]	6.70[g]	0.24[f]
15	12.72[f]	1.83[g]	5.01[fg]	0.24[f]
20	11.89[f]	0.92[f]	3.94[f]	0.17[f]
25	11.20[f]	0.61[f]	3.57[f]	0.14[f]

[a] Means within a column having different superscripts are significantly different ($P < 0.05$), n = 30.
[b] % cookloss = (sample wt − cooked emulsion wt) × 100/sample wt.
[c] mL fat/100 g emulsion = fat released × 100/sample wt.
[d] mL gel/100 g emulsion = ml gel released × 100/sample wt.
[e] wt solids released × 100/sample wt.

emulsions made with SIF might have been due to enhanced microbial growth through greater nutrient content, quality, or availability. Craven and Mercuri (1977) observed that two of four chicken patties, extended with texturized soy, had higher aerobic plate counts than the all-meat control. Busta and Schroder (1971) suggested that soy proteins might contain some unidentified growth factor(s) that enhance microbial growth over that observed in meat preprations. In this study, the average psychorotrophic log count for the reference all-meat emulsion, 3.88, was lower than the log counts from emulsions containing SCF or SIF (Table 4), supporting the observations of Craven and Mercuri (1977). However, the average mesophilic count for the all-meat reference emulsion was higher than that of the SCF of VPF emulsions, but lower than the SIF emulsion.

The cooking procedure reduced the aerobic plate count ($7°C$) from 5.24 to < 2.00 and the count at $35°C$ from 5.41 to 2.43. During the first 2 wk of storage of the cooked emulsions, however, both psychrotrophic ($7°C$ and $20°C$) and mesophilic ($35°C$) log counts were significantly ($P < 0.05$) increased (Table 4). The growth phase for the two types of organisms represented by the different incubation temperatures probably occurred at two different times during the initial 2 wk of storage. Since the mesophilic growth appeared to be arrested after only 2 wk of storage (Table 4) due to the low temperature, it could be concluded that the increase in growth observed during the first 2 wk occurred during the cool-down period within a short time after cooking. According to Jay (1978), psychrotrophic organisms tend to have a longer lag phase and a slower growth rate than mesophiles. It could be concluded that the psychrotrophic growth phase occurred late in the first 2 wk of storage, since log counts ($7°C$ and $20°C$) gradually increased over the remaining storage period (Table 4).

Emulsion coliform counts were found to be < 10 cfu/g after cooking and remained at that level throughout the storage period, regardless of treatment (data not shown).

Rehydrated flour level had no significant ($P < 0.05$) effect on the DWL, hue, of the cooked sausages (Table 5); however, incorporation of VPF or SCF resulted in significantly ($P < 0.05$) redder sausages (higher DWL) than those

containing SIF. DWL appeared to increase with storage, possibly due to dehydration, and thus, pigment concentration, since the casings were not air tight. Sausages incorporating the 25% level of rehydrated flour had a significantly ($P < 0.05$) higher EP (intensity) than the 15% or 20% levels, but not significantly ($P > 0.05$) different from the 10% level (Table 5). Rehydrated flour level appeared to have little effect on the LUM (brightness) of the products. The lack of a consistent trend in these data was probably an artifact resulting from the flour type x storage interaction.

The significant ($P < 0.05$) interactions of flour type by storage for LUM and EP are presented in Fig. 1 and 2, respectively. Addition of VPF generally produced a lighter product (higher LUM) than those incorporating SCF or SIF at all storage times (Fig. 1). Storage produced darker (lower LUM) VPF and SIF emulsions; however, SCF emulsions became lighter (higher LUM) with storage. Changes were small and thought to reflect DWL related storage changes.

VPF emulsions exhibited the least intense color (lowest EP), regardless of storage time (Fig. 2). During storage, there was a slight increase in EP for the VPF products, while SCF products tended to develop a slightly less intense color. SIF emulsions appeared to have rather static EP values as storage progressed.

Statistical analysis of sensory data parameters indicated that no real differences due to flour level or storage time could be detected (data not shown). This appeared to be a function of the rather large variation in panelist responses to the sensory attributes of the emulsions. All average values for all treatments tested fell within the "acceptable" category.

CONCLUSIONS

EMULSIONS prepared with VPF had better emulsion stability than those containing SCF or SIF. Flour type had little effect on the storage stability except that the incor-

Table 5—Dominant wavelength (DWL), excitation purity (EP), and luminosity (LUM) for emulsions prepared with vegetable protein flour (VPF), soy concentrate flour (SCF), or soy isolate flour (SIF)[a]

	DWL (nm)	EP (%)	LUM (%)
Rehydrated flour level (%)[b]			
10	582.48[e]	31.07[ef]	28.29[e]
15	582.48[e]	30.91[e]	29.08[ef]
20	582.62[e]	30.71[e]	29.49[f]
25	582.50[e]	31.56[f]	28.96[ef]
Flour type[c]			
VPF	582.62[e]	**	**
SCF	582.71[e]		
SIF	582.23[f]		
Storage time (wk)[d]			
Initial	582.15[e]	**	**
2	582.43[ef]		
4	582.63[f]		
6	582.66[f]		
8	582.74[f]		

[a] Means within a column and a section having different superscripts are significantly different ($P < 0.05$).
[b] n = 40.
[c] n = 30.
[d] n = 24.
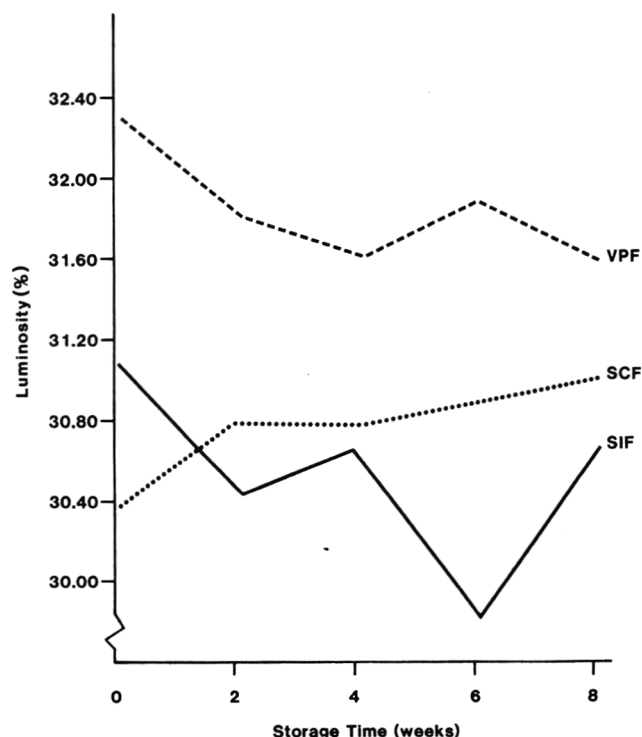** Significant interaction with storage (see Fig. 1 and 2).

Fig. 1—Percent luminosity (LUM) during storage for emulsions prepared with vegetable protein flour (VPF), soy concentrate flour (SCF), or soy isolate flour (SIF).
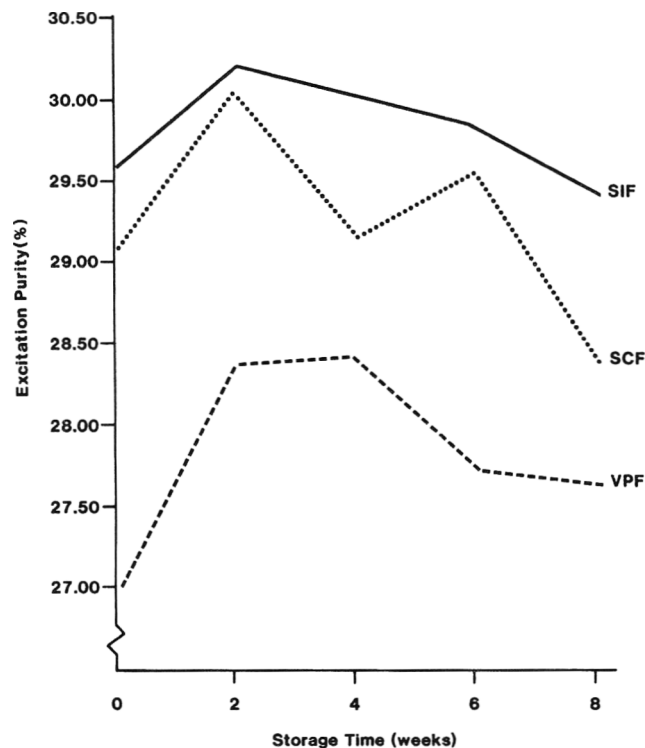
Fig. 2—Percent excitation purity (EP) during storage for emulsions prepared with vegetable protein flour (VPF), soy concentrate flour (SCF), or soy isolate flour (SIF).

poration of SIF produced higher mesophillic and psychrotrophic counts in the product after 2 wk of storage. This might indicate that SIF products would have a shorter shelf life than products incorporating VPF or SCF. Emulsions made with SIF produced a less red and less intense color than SCF or VPF emulsions.

Flour level had no effect on rancidity, aerobic plate counts, DWL, or LUM; however, increased flour levels produced better emulsion stability (20% and 25%), lowered EP, and resulted in less firm emulsions.

Increased storage time caused increased aerobic plate counts, rancidity values, tensile strength, and DWL.

All emulsions were rated as acceptable by the sensory panelists. Panelists could detect no real differences between flour level within a storage time.

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Factors Affecting Properties of Raw-Frozen Pork Sausage Patties Made with Various NaCl/Phosphate Combinations

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ABSTRACT

Whole hog sausage patties made with (1) no phosphate, (2) 0.375% sodium tripolyphosphate (STP) and (3) 0.441% Lem-O-Fos® in combination with salt levels of 1.5, 1.0, 0.5, and 0.0% NaCl were frozen and packaged. Use of phosphates decreased off-flavor and rancidity development, improved binding, and increased pH values, cooking yields and scores for saltiness and juiciness. The prooxidant effect of NaCl was masked by the antioxidant properties of STP and Lem-O-Fos®. Extended periods of frozen storage reduced cooking yields, juiciness and texture scores and enhanced development of off-flavor and rancidity. However, use of phosphates or vacuum packaging exhibited an antioxidant effect during extended periods of frozen storage.

INTRODUCTION

THE UNITED STATES produces over 500 million kg of fresh (not cured) sausage under Federal Inspection each year and pork sausage represents as much as 80% of this total (AMI, 1982). Per capita consumption of processed meats in the U.S. is about 23 kg (AMI, 1982); however, reports linking excessive sodium intake to the incidence of hypertension (Hunt, 1981) may cause a decline in the consumption of processed meats. The majority of sodium in the American diet comes from processed foods (Knauer, 1981), mostly in the form of sodium chloride (NaCl). The functional role of NaCl in processed meats has been reviewed by Terrell (1983). There appear to be two approaches to reducing the sodium content of processed meats: (a) reduction of sodium chloride, or (b) replacement of sodium chloride with other chloride salts. Olson (1982) reported that a 25% reduction in NaCl is probably the most that can be achieved without detrimentally affecting product characteristics (flavor, texture, shelf-life). Recent studies on reduction or replacement of NaCl in processed meats suggest that replacement of NaCl with other chloride salts may not be totally desirable for flavor and that reducing levels of NaCl may affect the shelf-stability of such products (Olson and Terrell, 1981).

Use of alkaline phosphates may be the most practical alternative when reducing salt levels in processed meats (Rust and Olson, 1982). A recent ruling by USDA-FSIS (1982) allows the expanded use of sodium or potassium phosphates (0.5% level) in certain processed meat products. Phosphates are known to increase water-holding capacity, stabilize meat emulsions, improve juiciness and tenderness, and maintain flavor of processed meat products (Ellinger, 1972). Reduction of the sodium content in processed meats could be achieved by reducing NaCl levels in the presence of sodium phosphates, since NaCl contains about 40% sodium and most sodium phosphates contain about 30%

sodium. Accordingly, the aim of the current research was to determine effects of reduced NaCl levels, reduced NaCl levels in combination with the addition of sodium tripolyphosphate (STP) and reduced NaCl levels in combination with the addition of Lem-O-Fos® (STP and a natural antioxidant) on properties of raw pork sausage patties during extended periods of frozen storage.

MATERIALS & METHODS

Sausage manufacture

Five sows (live weight 116 – 186 kg) were slaughtered and fabricated (24 hr postmortem) into boneless portions of fat and lean meat. These portions were ground separately through a 3-hole kidney shaped plate and combined to obtain 36 batches (7.26 kg each) of sausage meat with a targeted fat level of 35%. These formulated meat batches were packaged in polyethylene-coated freezer paper and frozen at −34°C prior to making pork sausage. For each repliction of the experiment, 12 frozen meat batches were thawed at 2°C overnight to a temperature of −3 to −2°C. Three replications of the 12 ingredient combinations were made at 3-day intervals to contain 0.375% sodium tripolyphosphate (STP), 0.441% Lem-O-Fos® and control or no phosphate in combination with salt levels of 1.5, 1.0, 0.5 and 0.0% NaCl for each phosphate group (3 × 4 factorial design). Lem-O-Fos® (Stauffer Chemical Co., Ft. Washington, PA) is a commercial blend of STP and natural lemon juice solids. In order to maintain a STP content equivalent to that of STP alone, a higher level of Lem-O-Fos® was used. In addition to the appropriate amounts of NaCl and phosphate, each ingredient combination contained 3% ice water and 61.4g of seasoning. The seasoning consisted of sage, black pepper, sugar, monosodium glutamate and cracked red pepper. After mixing all ingredients, the sausage was reground through a plate with 4.76 mm holes. The product was then mixed 3 min, by hand, and stuffed (pneumatic piston stuffer) into fibrous casings (6.985 cm diam and 61 cm length). Logs of sausage were frozen (−34°C) for 4 hr and then held overnight at −9°C. Logs were tempered to −3°C before slicing into 9.5 mm thick patties with a Hobart Model 420 slicer.

Packaging and storage

Patties were placed in plastic foam boat trays (8 patties/tray) and packaged using either oxygen permeable polyvinyl chloride (PVC) film overwrap or vacuum packaged (VP) in oxygen impermeable Saran-polyethylene bags (Curwood, Inc., New London, WS) and assigned to frozen (−9°C) storage periods of 0, 2, 4 or 8 wk.

Analytical methods

At the 0-wk storage period, samples from all 3 replications were removed for determination of moisture, fat and NaCl (AOAC, 1980) and pH, using 10g meat/100 mL distilled-deionized water, measured with an Orion Research Model 801A digital ionalyzer. Samples from the first replication were removed at 0, 4 and 8 wk of frozen storage to determine the extent of rancidity development (lipid oxidation) by the thiobarbituric acid (TBA) procedure described by Rhee (1978). Results were expressed as TBA number (mg malonaldehyde/kg sample).

Sensory evaluation and cooking procedure

A sensory panel was trained for a period of 1 wk (two sessions per day) using patties formulated from a trial replication of this experiment. Panelists assisted in constructing the descriptive rating scales used to evaluate the patties. A 10-member panel was selected based on the ability of the panelists to discriminate and reproduce

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results. This trained panel evaluated the patties according to the following traits and scales: texture, 8 = extremely crumbly, 1 = extremely not crumbly; juiciness, 8 = extremely juicy, 1 = extremely dry; saltiness, 8 = extremely salty, 1 = extremely not salty; and off-flavor, 8 = extremely weak to none, 1 = extremely strong. Patties were randomly assigned, within replication, to sensory panel sessions at 0, 2, 4 and 8 wk of frozen storage. Two panel sessions per day were conducted during a 3-day period for each replication and storage period. Raw patties were fried, from the frozen state, in electric skillets set at 107°C for approximately 4 min per side until a product temperature of 71 – 74°C (measured with thermocouples) was achieved. Weights before and after skillet frying were obtained to determine cooking yield. Patties were sectioned (one-half patty per panelist) and served, while warm, to the panelists. Panelists were served in a sensory panel laboratory equipped with partitioned booths and controlled levels of incandescent light. Panelists evaluated eight samples per session and were served water (room temperature) for rinsing between samples.

Statistical analysis

The statistical model was a split-split plot design. Main plots were represented by the 12 ingredient combinations, arranged in a 3×4 factorial consisting of 3 phosphate groups and 4 levels of NaCl. Sub-plots were represented by packaging method and sub-sub plots represented by storage periods. Analysis of variance (SAS Institute, Inc., 1982) was used to test main effects and interactions. Means for main effects and interactions were separated using Least Significant Difference (Steel and Torrie, 1980).

RESULTS & DISCUSSION

MAIN EFFECT MEANS for properties of raw-frozen sausage patties are presented in Table 1. Significant interaction means are presented in subsequent tables, as indicated in this discussion.

Moisture, fat and NaCl content

Moisture and fat content were not significantly affected by salt level or phosphate group. As expected, NaCl content was affected ($P < 0.0001$) by the level of NaCl added to the sausage formulation. The NaCl content was also affected ($P < 0.05$) by phosphate group; those patties that contained phosphates (STP or Lem-O-Fos®) had a slightly higher NaCl content than patties made without phosphates.

Saltiness

Higher NaCl levels resulted in significantly higher saltiness scores. Saltiness scores were also increased ($P < 0.01$) with the use of phosphates. Saltiness scores were affected ($P < 0.05$) by storage period; this may be due to declines in cooking yield and juiciness that occur in raw-frozen patties over extended periods of storage. Packaging method did not affect ($P > 0.05$) saltiness scores.

pH

Significant interaction means for pH stratified by phosphate group and salt level are shown in Table 2. Within each phosphate group, increased levels of NaCl tended to raise pH values. Regardless of NaCl level, use of phosphates resulted in significantly higher pH values. Previous studies (Shults et al., 1972; Shults and Wierbicki, 1973) have shown that alkaline phosphates are effective for raising the pH of meat products, thereby increasing the water-holding capacity (WHC) of such products. Higher pH values have been associated with higher cooking yields, improved juiciness and greater bind in fresh pork sausage patties (Keeton, 1983).

Cooking yield

Significant interaction means for cooking yield stratified by phosphate group and salt level are shown in Table 2. Within each phosphate group, increased levels of NaCl tended to increase cooking yield. Sofos (1983) found that higher NaCl content and higher pH values were directly related to higher cooking yield for frankfurters made with beef and pork blends. At each NaCl level, use of phosphates resulted in a higher cooking yield than for patties made without phosphates. The results of this interaction are closely associated with the results found for pH in raw-frozen patties. Higher levels of NaCl and/or the use of alkaline phosphates are effective for increasing the pH of raw-frozen patties, thus contributing to higher cooking yields. Cooking yield was also affected ($P < 0.0001$) by storage period (Table 1). Extended periods of frozen storage resulted in lower cooking yield. Reagan et al. (1983) found increased cooking yields for fresh pork sausage patties

Table 1—Main effect means for properties of raw-frozen sausage patties[a]

Treatments	Moisture (%)[b]	Fat (%)[b]	NaCl (%)[b]	Saltiness[c]	pH[b]	Cooking[d] yield (%)	Juiciness[c]	Texture[c]	TBA no.[e]	Off-flavor[c]
Salt level										
1.5% NaCl	55.95[f]	32.91[f]	1.49[f]	6.0[f]	*	*	5.8[f]	5.0[h]	1.25[f]	*
1.0% NaCl	56.29[f]	34.29[f]	0.99[g]	5.1[g]	*	*	5.7[fg]	5.4[g]	1.82[f]	*
0.5% NaCl	56.96[f]	33.14[f]	0.56[h]	4.0[h]	*	*	5.4[g]	5.3[g]	1.25[f]	*
0.0% NaCl	57.07[f]	34.19[f]	0.13[i]	1.8[i]	*	*	5.0[h]	5.9[f]	1.06[f]	*
Phosphate group										
No phosphate	56.78[f]	33.62[f]	0.77[g]	3.9[g]	*	*	5.1[g]	5.6[f]	*	*
STP	56.04[f]	33.56[f]	0.79[fg]	4.4[f]	*	*	5.7[f]	5.3[g]	*	*
Lem-0-Fos®	56.88[f]	33.72[f]	0.81[f]	4.4[f]	*	*	5.7[f]	5.3[g]	*	*
Storage (wk)										
0				4.2[fg]		66.8[f]	6.0[f]	5.6[f]	*	*
2				4.2[fg]		63.7[g]	5.6[g]	5.2[g]	*	*
4				4.4[f]		61.5[h]	5.2[h]	5.4[fg]	*	*
8				4.1[g]		60.8[h]	5.2[h]	5.4[fg]	*	*
Packaging										
Vacuum packaged				4.2[f]		62.9[f]	5.6[f]	5.5[f]	*	*
PVC-wrapped				4.2[f]		62.7[f]	5.4[g]	5.3[f]	*	*

[a] * indicates interaction occurred.
[b] n=9 for salt level, n=12 for phosphate group.
[c] Sensory scales were: saltiness, 8=extremely salty, 1=extremely not salty; juiciness, 8=extremely juicy, 1=extremely dry; texture, 8=extremely crumbly, 1=extremely not crumbly; off-flavor, 8=extremely weak to none, 1=extremely strong. n=72 for salt level and storage period, n=96 for phosphate group, n=144 for packaging.
[d] n=60 for storage period, n=120 for packaging.
[e] mg malonaldehyde/kg. n=36 for salt level.
[fghi] Means within a column for each treatment followed by a common letter superscript are not different ($P > 0.05$).

stored at $-18°C$ for up to 28 days, but this study involved only one packaging method (commercial opaque sausage bags). Keeton (1983) found no effect due to storage for cooking yield of fresh pork sausage patties stored at $3°C$ (not frozen) for up to 21 days in either air-permeable film or vacuum packaged pouches. Cooking yield was not affected $(P > 0.05)$ by packaging method (Table 1).

Juiciness

Juiciness scores were affected $(P < 0.01)$ by NaCl level and phosphate group, as higher NaCl content or the addition of phosphates resulted in higher juiciness scores. This effect is obtained through the higher WHC that results when pH is elevated by the use of alkaline phosphates and/or higher NaCl content. Juiciness scores were also affected $(P < 0.0001)$ by storage period, as these scores declined during extended periods of frozen storage. Reagan et al. (1983) found that juiciness scores for fresh pork sausage patties declined after 21 days of frozen storage $(-18°C)$. Vacuum packaging improved $(P < 0.02)$ juiciness scores over those scores for patties wrapped in PVC. Vacuum packaging has been shown to be effective for maintaining quality in pork products during extended periods of storage (Smith et al., 1974).

Texture

Texture scores were affected by NaCl level $(P < 0.001)$ and phosphate group $(P < 0.02)$. Decreased NaCl levels resulted in higher texture scores, indicating less bind. Sodium chloride has been shown to be effective for improving the binding properties of processed meats (Terrell, 1983). The use of phosphates resulted in lower texture scores; alkaline polyphosphates are known to increase the solubility of meat proteins, allowing for greater WHC and increased bind (Ellinger, 1972). The effect of NaCl and alkaline phosphates on the textural properties of raw-frozen patties can be attributed to their influence on pH. Trout (1982) found that the addition of alkaline phosphates or the use of higher NaCl levels in restructured beef rolls resulted in higher values for pH and higher tensile strength, indicating greater bind. Texture scores were also affected $(P < 0.0001)$ by length of storage period; this may be due to declines in cooking yield and juiciness that also occur in raw-frozen patties during extended periods of frozen storage. Juiciness has been acknowledged as a contributing factor to the perception of texture or tenderness in a meat product. Packaging method did not affect $(P > 0.05)$ texture scores.

TBA number

TBA values were not affected $(P > 0.05)$ by NaCl level. Previous studies (Judge and Aberle, 1980; Rhee et al., 1983) have shown that increased NaCl levels enhance the development of oxidative rancidity (higher TBA number) in pork products. However, in the current study, the pro-oxidative effect of NaCl may have been masked by the antioxidant properties of the sodium phosphates. This effect will be explained in the following discussion. Significant interaction means for TBA number stratified by storage period and phosphate group are shown in Table 3. Within storage periods, means were not different $(P > 0.05)$ at 0 wk, regardless of phosphate group. However, at the 4 and 8 wk storage periods, those patties made with phosphates had significantly lower TBA values than patties containing no phosphate. Within phosphate groups, patties containing no phosphate had significantly higher TBA values at each successive storage period. Patties made with STP or Lem-O-Fos® did not increase $(P < 0.05)$ in TBA number until the 8 wk storage period. This antioxidant effect of alkaline phosphates has been attributed to their ability to complex prooxidant metal ions (Ellinger, 1972) and also appears to be related to the increased pH values associated with their use in meat products. Other workers (Judge and Aberle, 1980; Drerup et al., 1981) have found decreased TBA values directly related to higher pH values in fresh pork products. Although NaCl alone may increase pH, the effect is not as great as that achieved by the addition of alkaline phosphates; this may account for the fact that NaCl level did not significantly affect TBA values in this study. Significant interaction means for TBA number stratified by storage period and packaging are shown in Table 4. Within storage periods, means were not different $(P > 0.05)$ at 0 wk, regardless of packaging method. However, at the 4 and 8 wk storage periods, the use of vacuum packaging significantly lowered TBA values. Within packaging methods, vacuum packaged patties did not increase $(P > 0.05)$ in TBA number throughout the entire storage period. However, PVC-wrapped patties significantly increased in TBA number at each successive storage period. This interaction indicates that vacuum packaging is an ef-

Table 2—Significant interaction means for pH, cooking yield and off-flavor scores stratified by phosphate group and salt level

	pH[a]	Cooking yield (%)[b]	Off-flavor[c]
No phosphate			
1.5% NaCl	5.68[h]	62.6[ghi]	6.4[ef]
1.0% NaCl	5.59[i]	59.7[j]	6.0[g]
0.5% NaCl	5.60[hi]	60.8[ij]	6.5[de]
0.0% NaCl	5.41[j]	60.1[j]	6.1[fg]
STP			
1.5% NaCl	6.05[e]	64.6[ef]	6.8[d]
1.0% NaCl	6.14[d]	63.7[fg]	6.8[d]
0.5% NaCl	6.04[e]	63.0[fgh]	6.8[d]
0.0% NaCl	5.92[g]	61.2[hij]	6.8[d]
Lem-0-Fos®			
1.5% NaCl	6.05[e]	66.6[d]	6.7[de]
1.0% NaCl	6.01[ef]	62.2[ghi]	6.8[d]
0.5% NaCl	5.94[fg]	65.8[de]	6.5[de]
0.0% NaCl	5.92[g]	63.3[fg]	6.6[de]

[a] n=3.
[b] n=20.
[c] 8=extremely weak to none, 1=extremely strong. n=24.
[defghij] Means within a column followed by a common letter superscript are not different (P>0.05).

Table 3—Significant interaction means for TBA number and off-flavor scores stratified by storage period and phosphate group

	TBA no.[a]	Off-flavor[b]
0 wk		
No phosphate	0.44[g]	7.1[cd]
STP	0.22[g]	7.2[c]
Lem-0-Fos®	0.25[g]	7.1[cd]
2 wk		
No phosphate		6.3[g]
STP		6.8[de]
Lem-0-Fos®		6.8[de]
4 wk		
No phosphate	2.40[d]	6.2[g]
STP	0.88[fg]	6.7[ef]
Lem-0-Fos®	0.73[fg]	6.5[efg]
8 wk		
No phosphate	3.89[c]	5.4[i]
STP	1.76[de]	6.4[fg]
Lem-0-Fos®	1.56[ef]	6.2[g]

[a] mg malonaldehyde/kg. n=16.
[b] 8=extremely weak to none, 1=extremely strong. n=24.
[cdefgh] Means within a column followed by a common letter superscript are not different (P > 0.05).

Table 4—Significant interaction means for TBA number and off-flavor scores stratified by storage period and packaging

	TBA no.[a]	Off-flavor[b]
0 wk		
Vacuum packaged	0.30[e]	7.2[c]
PVC-wrapped	0.30[e]	7.1[c]
2 wk		
Vacuum packaged		6.7[d]
PVC-wrapped		6.5[de]
4 wk		
Vacuum packaged	0.82[e]	6.6[d]
PVC-wrapped	1.86[d]	6.3[e]
8 wk		
Vacuum packaged	0.55[e]	6.5[de]
PVC-wrapped	4.26[c]	5.5[f]

[a] mg malonaldehyde/kg. n=24.
[b] 8=extremely weak to none, 1=extremely strong. n=36.
[cdef] Means within a column followed by a common letter superscript are not different (P>0.05).

Table 5—Significant interaction means for off-flavor stratified by phosphate group and packaging

	Off-flavor[a]
No phosphate	
Vacuum packaged	6.6[b]
PVC-wrapped	5.8[c]
STP	
Vacuum packaged	6.9[b]
PVC-wrapped	6.7[b]
Lem-0-Fos®	
Vacuum packaged	6.8[b]
PVC-wrapped	6.5[b]

[a] 8=extremely weak to none, 1=extremely strong. n=48.
[bc] Means within a column followed by a common letter superscript are not different (P > 0.05).

fective method to retard the development of rancidity in raw-frozen sausage patties.

Off-flavor

Significant interaction means for off-flavor scores stratified by phosphate group and salt level are shown in Table 2. Within phosphate groups, patties made with STP or Lem-O-Fos® were not different (P > 0.05) in off-flavor scores, regardless of NaCl level. Off-flavor scores for patties containing no phosphate were affected (P < 0.05) by NaCl, but no clear pattern was established. At each NaCl level, patties made with phosphates had higher off-flavor scores (less off-flavor) than patties made with no phosphate. This interaction substantiates the findings for TBA number of raw-frozen patties, which suggest that alkaline phosphates may mask the prooxidant effect of NaCl. Significant interaction means for off-flavor scores stratified by storage period and phosphate group are shown in Table 3. Within storage periods, patties did not differ (P > 0.05) in off-flavor scores at the 0 wk, regardless of phosphate group. However, at subsequent storage periods, patties made with phosphates had less off-flavor than patties containing no phosphate. Within phosphate groups, patties at the 8 wk storage period had more off-flavor (P < 0.05) than patties stored for 0 to 2 wks, regardless of phosphate group. For patties made with phosphates, off-flavor scores at the 8 wk storage period were not different (P > 0.05) from scores at 4 wks of storage. However, patties containing no phosphate had significantly more off-flavor at 8 wks of storage than at 4 wk. This interaction also supports previous results in this study which indicate that an antioxidant effect is achieved through the use of phosphates. Significant interaction means for off-flavor scores stratified by storage period and packaging are shown in Table 4. Within storage periods, off-flavor scores did not differ (P > 0.05) at 0 or 2 wks of storage, regardless of packaging method. The use of vacuum packaging significantly improved off-flavor scores at 4 and 8 wk of storage. Within packaging methods, off-flavor scores declined (P < 0.05) beyond the 0 wk storage period, regardless of packaging method. Vacuum packaged patties were not different (P > 0.05) in off-flavor scores at 2, 4 or 8 wk of storage. However, PVC-wrapped patties had significantly more off-flavor at 8 wks of storage than they did at 2 or 4 wk. This interaction indicates that vacuum packaging is effective for controlling the development of off-flavors in raw-frozen sausage patties during extended periods of frozen storage. Significant interaction means for off-flavor scores stratified by phosphate group and packaging are shown in Table 5. Within phosphate groups, patties containing no phosphate

had significantly less off-flavor when vacuum packaged than when they were wrapped with PVC. Patties made with phosphate did not differ (P > 0.05) in off-flavor scores, regardless of packaging method. Within packaging methods, the use of phosphates did not affect (P > 0.05) off-flavor scores for vacuum packaged patties. However, PVC-wrapped patties had less off-flavor (P < 0.05) when they were made with either STP or Lem-O-Fos®. This interaction suggests that it is not necessary to utilize both vacuum packaging and the addition of phosphates to achieve an antioxidant effect in raw-frozen sausage patties. The singular effect of either phosphates or vacuum packaging is enough to control the development of off-flavors.

CONCLUSIONS

THE REDUCTION of NaCl content from 1.5% to 1.0%, along with the use of 0.375% STP, would allow a reduction (approximately 15%) of the sodium content in raw-frozen sausage patties without detrimentally affecting the sensory characteristics or cooking yield of this product. This study shows that the use of STP or Lem-O-Fos® increased pH and binding, cooking yield, saltiness and juiciness scores while protecting against the development of off-flavor and rancidity. These important "technical effects" would compensate for the loss of functionality that occurs when NaCl levels are reduced. However, a minimum level of NaCl (approximately 1.0%) is necessary to retain flavor, cooking yield and product characteristics in raw-frozen sausage patties.

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Effect of Carbon Dioxide Flushing and Packaging Methods on the Microbiology of Packaged Chicken

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─────────ABSTRACT─────────

Dry packed broilers were individually packaged by carbon dioxide flushing, vacuum packaging and conventional stretch wrapping in air. The chicken was stored in a display case at about 5°C and examined for various bacteria, odor and slime development at intervals up to 17 days. Carbon dioxide flushing resulted in longest shelf life, with vacuum packaging next, and stretch wrapping in air least desirable. CO_2 packaging also produced a unique "snugging" effect resembling vacuum packaging. Location of chicken at the top or bottom of the pack also was observed for differences in microbiological quality, but no significant differences were observed.

INTRODUCTION

VACUUM PACKAGING of poultry and other meats has been used successfully in preserving these produces because most spoilage organisms are mainly aerobic and exclusion of air inhibits their growth (Wells et al., 1958; Shank and Lundquist, 1963; Rey and Kraft, 1971; Debevere and Voets, 1973; Arafa and Chen, 1975). Although vacuum packaging is being increasingly used as an adjunct to packaging fresh meats, particularly primal cuts, it is surprising that less consideration has been given to packaging retail poultry, which is even more perishable than red meat items. In earlier work done in our laboratory, we found that growth of pseudomonads and bacterial fluorescence was inhibited on chicken packaged in evacuated heat shrunk Cryovac bags (Kraft and Ayres, 1961). Later, we observed that whole or cut-up chicken had the longest shelf-life when a high barrier film was used for vacuum packaging compared with a low barrier material or with conventional packaging in air (Kraft et al., 1982).

However, concern still exists that vacuum packaging may change the "normal" microbial flora so that organisms having less aerobic characteristics and which are potential pathogens might outgrow the typical aerobic spoilage types, thus producing a hazard that otherwise would not exist. Also, vacuum packaging produces a change in the typical spoilage flora from *Pseudomonas* species, which may produce extensive proteolysis or lipolysis, to more facultative "souring" types of bacteria such as *Lactobacillus, Microbacterium*, and enteric bacteria. The possibility also exists that staphylococci and facultative anaerobes may be favored by a more anaerobic environment than occurs with packaging in an atmosphere of air. Spoilage from organisms that may grow on vacuum packaged meats, however, is not as extensive or pronounced as that occurring from activity of aerobic spoilage types. Potential pathogens, such as salmonellae, *C. perfringens*, and *Staphylococcus aureus* do not ususally present a hazard in refrigerated vacuum packaged meats since refrigeration inhibits their growth (Holland, 1980).

Modified atmospheres, in which a pre-determined gaseous atmosphere is flushed into a gas impermeable package, have been used to extend keeping time by inhibiting microbial growth on meats. Gases include carbon dioxide (CO_2), oxygen (O_2), and nitrogen (N_2). Modified atmosphere packaging provides a complex enviroment in the package. In addition to respiration by the meat and consumption of oxygen by microorganisms, carbon dioxide also may diffuse into the meat (Ogilvy and Ayres, 1951; Seideman et al., 1979). The gas extends the lag phase and generation time of bacteria, thus inhibiting growth.

Many investigations could be cited on the effects of CO_2 on various types of fresh meats, but the concern here is with poultry and extension of shelf life of broilers. One of the earliest studies of the application of CO_2 to preservation of poultry was done by Smith (1934) in which he reported extensive discoloration of exposed flesh of carcasses in the presence of high concentrations of carbon dioxide. Ogilvy and Ayres (1951) reported that a maximum concentration of 25% carbon dioxide could be used without discoloration developing but with extension of storage life.

For several years, the poultry industry has been making use of CO_2 to help preserve bulk packed products during transporatation. One system involves addition of CO_2 gas to broilers packed in a plastic film bag in a fiberboard box. This system and an evaluation of controlled atmospheres for tray-packaging of poultry was recently reviewed by Moore (1984). The primary purpose of the present study was to determine if extended storage life could be obtained by applying the CO_2 "flush" to retail packages of individual carcasses after previous bulk pack treatment such as that described. Additional work was directed at determining if diferences in microbiological quality occurred among chickens because of location in the bulk pack.

MATERIALS & METHODS

EVISCERATED DRY PACKED whole broilers, approximately 70 lb or about 20-25 birds per box, were obtained from local commercial distributors within 48 hr after processing, and had been shipped by refrigerated truck. The poultry originally was flushed with CO_2 in the bulk package by means of a system similar to that described by Moore (1984). One-half of the carcasses were cut up in the laboratory and the remainder were packaged as whole birds. For each of these cutting treatments, three packaging methods were used. One third of the birds were "stretch wrapped" in air, one-third were vacuum packaged, and the last group was flushed with commercial carbon dioxide. The atmosphere in the vacuum packages was reduced to 28-29 in. Hg, or as completely evacuated as possible with the high vacuum equipment used. For the CO_2-packed birds, the packages were first evacuated, then sufficient CO_2 was added to return the pressure to about 50% atmospheric pressure (14-15 in. Hg). A high barrier film (oxygen permeability was 1 $cc/m^2/24$ hr at 24°C, 0% RH) was used for both vacuum packaging and CO_2 flushing. For the broilers manually tray packaged in air, the stretch wrap film had a permeability of 6500 $cc/m^2/24$ hr at 24°C and 0% RH. Following packaging, the poultry was stored in a display case at about 5°C and examined as described below at intervals of 0, 3, 6, 9, 13 and 17 days of storage. In another series of tests, chickens from the two layers in the fiberboard box were examined after similar display case storage. These birds also were received within 48 hr after processing from plants in the South. At each interval up to 10 days of storage in the display case, the surface and cavity of two carcasses originally from the top and two from the bottom layers in the box were sampled for microbiological analyses.

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Analyses were done for mesophilic and psychrotrophic (facultative psychrophilic) bacteria for typical low temperature spoilage types, facultative "souring" organisms such as lactobacilli, and potential pathogens including *Clostridium perfringens*, salmonellae and staphylococci as detailed in Table 1. All samples were evaluated for gross evidence of off-odors and slime formation by three observers. Odor and slime development was described as long ag as 1950 by Ayres et al. as a measure of spoilage, and has been used by many investigators since that time. Salmonellae isolates were serotyped at the State Hygenic Laboratory at the University of Iowa. Three replications were conducted, and the data were analyzed statistically by analysis of variance (Snedecor and Cochran, 1967). The SAS computer system was utilized to simplify calculations and evaluation of results.

RESULTS & DISCUSSION

Subjective of evaluation of shelf life

Vacuum-packaged chickens showed some evidence of yellowing of the skin. Fluid withdrawn from the carcass during the process and further accumulating during storage was visibly dispersed throughout the package. Both of these occurrences resulted in a product less attractive in appearance than the conventional stretch wrapped commercial product. The yellowness observed after vacuum packaging disappeared after the package was opened and the skin was exposed to air.

Vacuum-packaged birds were free of slime up through day 13 of storage. However, whole birds were judged unacceptable at day nine because of strong off-odors emanating from the visceral cavities. Both whole and cut-up birds had developed pronounced off-odors between the 9th and 13th day although these odors were less offensive than those from stretch-wrapped birds. The odors were indicative of the presence ammonia, similar to the description given by Ayres et al. (1950) as "ammoniacal" and characteristic of advanced spoilage.

The carbon dioxide treated birds appeared acceptable for as long as 20 days in the case of whole birds, then off odors were observed, and 17 days for cut-up broilers, which developed off odors by that time. Immediately after packaging, the packaging material fitted loosely around the product, but within 24 hours it was as tightly fitting as the vacuum-packed birds, but without the discoloration and drip. This "snugging" phenomenon may be explained by the absorption of carbon dioxide gas into the exposed surfaces of the chicken and the consequent solution of the gas in the tissues. The result was the creation of a vacuum within the package and a close fit of the plastic film around the product. No visible increase in size of birds due to gas absorption occurred.

Stretch-wrapped birds were judged to have unacceptable odors and appearance by the ninth day of storage. In general, the cavities had a stronger undesirable odor than did the surfaces, and the cut-up birds were hence less offensive than the whole birds. However, in addition to off-odors, there was also evidence of slime and fluorescence under ultraviolet light on stretch-wrapped birds by the ninth day of storage. Between day 9 and day 13 carcasses became covered with slime which was evidenced as a sticky layer on the skin surface.
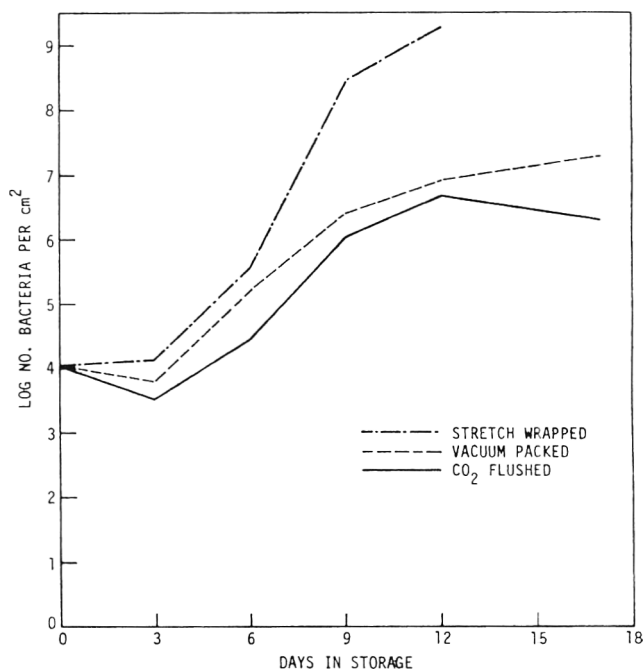
Fig. 1—Effect of packaging method on growth of mesophilic organisms on whole chickens.

Table 1—Bacteriological procedures employed to determine bacterial densities or incidence

Types of microorganisms	Growth media	Plating technique	Incubation	Confirmatory tests
Mesophiles	Trypticase Soy agar (TSA) (BBL)[b]	Surface plating[a]	30°C/24-48 hr	
Psychrotrophs	Trypticase Soy agar (BBL)	Surface plating	5°C/10-21 days	
Coliforms	Violet Red Bile (VRB) agar (Difco)[c]	Surface plating/ VRB overlay	37°C/24-48 hr	
Lactobacilli	LBS agar (BBL)	Surface plating	30°C/ 5-10 days	Gram stain
Staphylococci	Staph 110 medium with egg yolk (Herman and Morelli, 1960)	Surface plating	37°C/48 hr	Tube coagulase test
Clostridium perfringens	SPS agar (Angelotti et al., 1962)	Anaerobic pouches (Bladel and Greenberg, 1965)	37°C/24 hr	Motility and H₂S (Angelotti et al., 1962)
Salmonella[d]	Procedures for meats (Galton et al., 1968)	Procedures for meats (Galton et al., 1968)	37°C/24 hr	TSI, LIA, and agglutination (Galton et al., 1968)

[a] Surface plating technique of Thomas et al. (1981).
[b] BBL Division of Bio Quest, Cockeysville, MD.
[c] Difco Laboratories, Detroit, MI.
[d] Qualitative determination.

These findings are supported by an earlier study reported by Kraft and Ayres (1961) which showed that growth of fluorescent pigment-producing pseudomonads was inhibited by packaging in evacuated Cyrovac bags due to the exclusion of air. Since the stretch-wrapped packages were permeable to oxygen, it was expected that the predominant spoilage flora on the chicken in these packages would be pseudomonads, some of which were likely to produce fluorescent pigmetns.

Objective evaluation of shelf life

Stretch-wrapped birds had larger numbers of mesophilic organisms than either vacuum-packed or carbon dioxide-flushed birds. These organisms grew more rapidly and attained their largest numbers on stretch-wrapped chicken. Fig. 1 for whole chicken and Fig. 2 for cut-up broilers show the relationship between growth of mesophiles and the packaging process.
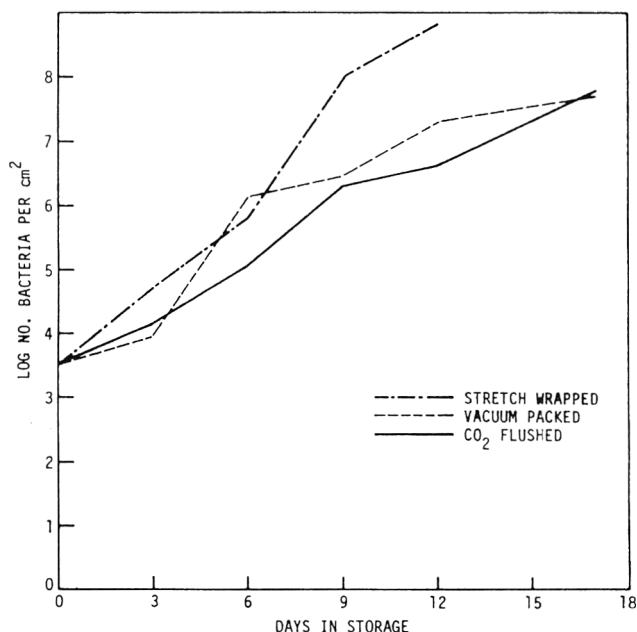
By the 9th day of storage, the mesophilic load was in excess of 250×10^6 organisms per sq cm on both whole and cut-up stretch-wrapped birds. Vacuum-packaged poultry reached mesophilic numbers of only 19×10^6 per sq cm on day 17. By comparison, mesophiles numbered only about 1.9×10^6 per sq cm on whole, carbon dioxide-flushed birds by day 17.

Psychrotrophs were considered to be organisms that grew well at low temperature, although their optimum growth temperature may be higher, as suggested by Eddy (1960). The incidence of psychrotrophs paralleled that for mesophiles, with similar relationships exhibited (Fig. 3 and 4).

The highest counts of coliform organisms were found on stretch-wrapped birds and by the 13th day of storage numbers were in excess of 100×10^6 per sq cm. Vacuum-packed birds had much lower counts than stretch-wrapped, and carbon dioxide-treated birds had the lowest counts. Few coliforms were found on whole, carbon dioxide-packed birds, numbering only 300 organisms per sq cm on day 17 of storage (Fig. 5).

Whenever lactobacilli were found, their numbers increased with storage time regardless of the packaging method used. However, in contrast to the results for total aerobes, stretch-wrapped birds had the lowest counts, reaching less than 200 lactobacilli per sq cm by day 13 of storage. The carbon dioxide-treated birds had more than 1.9×10^4 per sq cm by the 13th day of storage and vacuum-packed whole birds over 5×10^6.

Vacuum-packaging or carbon dioxide-treatment inhibited growth of organisms that would normally successfully compete with lactobacilli and so the latter proliferated in the environment provided. These findings agree with those of other researchers (Bailey et al., 1979) who found more than 90% of the bacteria present on spoiled carcasses packed in CO_2 atmospheres were lactobacilli; and the work by Barnes and Shrimpton (1968) who reported that *Microbacterium*

Fig. 2—Effect of packaging method on growth of mesophilic organisms on cut-up chickens.
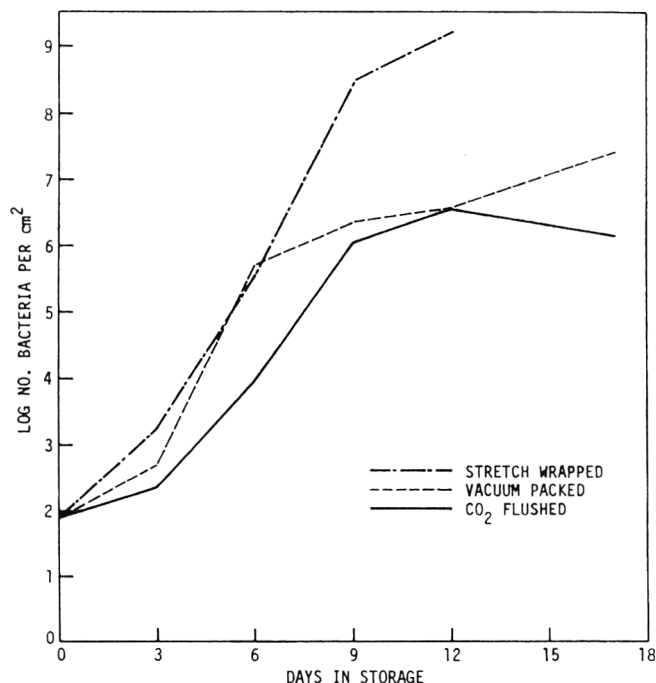

Fig. 3—Effect of packaging methods on growth of psychrotrophic organisms on whole chickens.
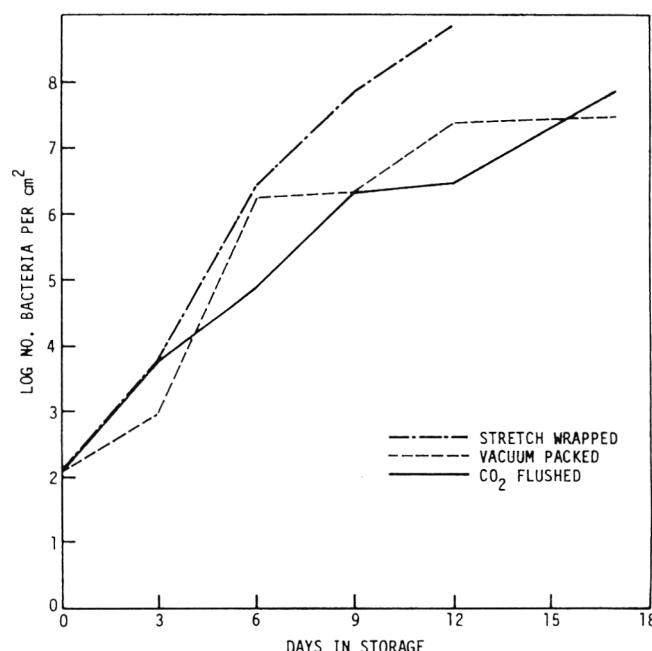

Fig. 4—Effect of packaging method on growth of psychrotrophic organisms on cut-up chickens.
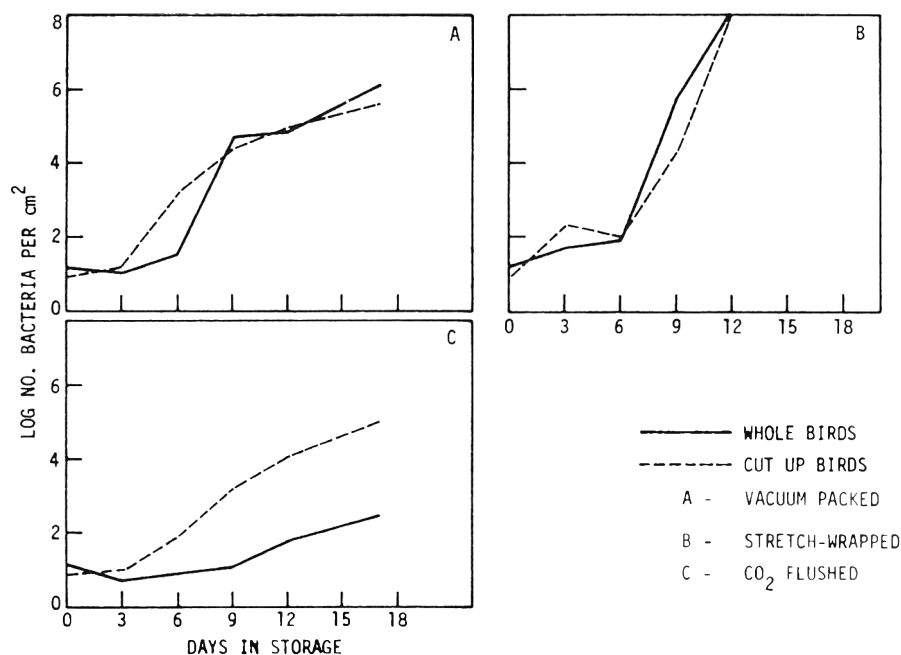
Fig. 5—Effect of packaging method on growth of coliforms on chicken.

——— WHOLE BIRDS
------ CUT UP BIRDS
A - VACUUM PACKED
B - STRETCH-WRAPPED
C - CO_2 FLUSHED

Table 2—Effect of packaging process and cutting on bacterial counts of chicken

| Treatments | Number of samples | Log no. bacteria per sq cm | | | | |
		Mesophiles	Psychro-trophs	Coliform	Lacto-bacilli	Staphylo-cocci
1. *Packaging process*						
CO_2 flushed	24	5.349[a]	5.028[a]	1.8635[a]	2.186[a]	1.251[a]
Vacuum packed	24	5.789[a]	5.530[a]	3.2408[b]	2.251[a]	1.216[a]
Stretch-wrapped	24	6.877[b]	6.691[b]	4.255[b]	1.403[a]	1.247[a]
2. *Type of poultry*						
Whole	36	5.891[a]	5.574[a]	2.852[a]	2.199[a]	0.820[a]
Cut-up	36	6.119[a]	5.925[a]	3.387[a]	1.695[a]	1.656[b]

[a,b] For each column counts having different letter superscripts are significantly different from each other at the 5% level.

thermosphactum and atypical lactobacilli formed the most significant part of spoilage flora on turkey carcasses packed in impermeable film.

The growth of coagulase-positive staphylococci on packaged poultry was very slow and only very low numbers were reached, usually before spoilage, followed by a decline in numbers up to the time of spoilage. Staphylococci are not good competitors and more rapid growth of spoilage organisms than staphylococci occurred at the low storage temperature. Cut-up birds generally had higher counts than whole birds probably as a result of increased handling of cut-up chicken and contamination of the product by human hands.

Very few incidences of other possible pathogens were evident, with only nine isolations of salmonellae of the 108 samples examined, and one instance of *Clostridium perfringens* during the course of the study. None of the three packaging methods presented undue hazard from the pathogens tested for in this work.

Statistical analysis of the data showed that there were significant differences among the three different packaging processes for the numbers of mesophiles, psychrotrophs, and coliforms (Table 2). The packaging methods were, however, not significantly different for growth of *Lactobacillus* and *Staphylococcus*. Table 2 presents mean counts for the entire storage period.

The analysis showed that the stretch-wrapped samples had significantly (p < 5%) higher counts for mesophiles and psychrotrophs than either vacuum-packed or carbon dioxide-flushed chickens. However, there was no significant

difference between the latter two packaging processes. On the other hand, the coliform counts on carbon dioxide-flushed birds were significantly (p < 0.05) lower than counts on either vacuum-packed or stretched-wrapped birds. As indicated, there was no significant difference between the packaging processes for growth of *Lactobacillus* or *Staphylococcus*.

Whole and cut-up birds showed no difference for all counts except for staphylococci which had significantly higher counts on cut-up birds because of the extra handling in the cutting operations.

In the second part of the study, there was no marked difference in microbiological quality between chickens from the top and chickens from the bottom of the bulk pack (Table 3). However, although total bacterial counts were lower in cavities, off-odors were generally stronger and more objectionable from cavities than from the surfaces of chickens.

The lack of difference in microbiological quality between chickens in different locations in the bulk pack container may be due to the "dry" packaging method as well as the CO_2 gas flush in the bulk pack. This process resulted in relatively little drip in the bottom of the box, and the carbon dioxide atmosphere within the pack successfully inhibited growth of organisms throughout the bulk package because of the ability of the gas to permeate the entire box. All product surfaces, regardless of location within the package, benefit from the inhibitory effects of carbon dioxide.

The inhibitory effect of carbon dioxide on growth of psychrotrophs including *Pseudomonas* which are the prin-

Table 3—Incidence of organisms on surface of bulk packed chickens (log no. organisms per sq cm)

Days of storage	Mesophiles		Psychrotrophs		Coliforms		Staphylococci	
	Top	Bottom	Top	Bottom	Top	Bottom	Top	Bottom
0	4.30	4.18	2.33	2.15	1.30	1.61	0.96	1.24
3	4.65	5.21	4.18	3.52	3.55	2.56	2.12	1.88
6	7.45	7.50	6.39	7.44	6.34	6.01	3.02	2.96
10	9.02	9.03	8.69	8.45	8.36	8.45	3.55	3.84

cipal spoilage organisms of refrigerated poultry, has been shown by other researchers. However, the present observations were made on the CO_2-flush process for retail poultry packaging and provide needed information regarding the process, compared with other packaging practices, for retail poultry.

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Factors Affecting Properties of Precooked-Frozen Pork Sausage Patties Made with Various NaCl/Phosphate Combinations

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—————————————ABSTRACT—————————————

Whole hog sausage patties made with (1) no phosphate, (2) 0.375% sodium tripolyphosphate (STP) and (3) 0.441% Lem-0-Fos® in combination with salt levels of 1.5, 1.0, 0.5 and 0.0% NaCl were precooked, frozen and packaged. Use of phosphates increased pH values, cooking yield, saltiness and juiciness scores and decreased the formation of off-flavor and rancidity. Higher NaCl levels increased saltiness scores and maintained juiciness scores and reheating yields over extended periods of frozen storage. Sodium chloride alone increased TBA values but did not affect (P > 0.05) off-flavor scores. The use of phosphates or vacuum packaging reduced the development of off-flavor and rancidity during extended periods of frozen storage.

INTRODUCTION

PORK SAUSAGE PATTIES find wide acceptance in the Hotel, Restaurant and Institutional (HRI) market because of their ease of preparation and their portioned size. Currently, precooked pork sausage patties are widely used in "Sausage N' Biscuit" sandwiches. The fact that pork sausage is highly susceptible to lipid oxidation and other alterations in quality during extended storage periods is well known (Judge and Aberle, 1980; Reagan et al., 1983). Sodium chloride (NaCl) promotes rancidity development (Rhee et al., 1983) but it is essential for product flavor and binding of the patties to achieve desirable texture (Terrell, 1983). In addition to these "technical effects" of NaCl in pork sausage patties, there is concern that excessive intake of sodium (Na) leads to hypertension (Dahl, 1958). As much as 50–75% of the average American's sodium intake comes from processed foods (Jacobson and Liebman, 1981), mostly in the form of NaCl. Accordingly, the meat processing industry has been encouraged to reduce the levels of NaCl used in formulating meat products (American Heart Association, 1978). Recent studies on reduction or replacement of NaCl in processed meats suggest that replacement of NaCl with other chloride salts may not be totally desirable for flavor and that reducing levels of NaCl may affect the shelf-stability of such products (Olson and Terrell, 1981). In addition to these reports, it is common knowledge that reducing NaCl levels may reduce product yields, an economic consideration.

A recent ruling by USDA-FSIS (1982), allowing the expanded use of sodium or potassium phosphates (0.5% level) in certain meat products, may offer a solution for reducing sodium contents in processed meats without a loss of product flavor and functionality. Reduction of the sodium content in processed meats could be achieved by reducing NaCl levels in the presence of sodium phosphates, because NaCl contains about 40% sodium and most sodium

phosphates contain about 30% sodium. Phosphates are known to increase water-holding capacity, stabilize meat emulsions, improve juiciness and tenderness, and maintain flavor of processed meat products (Ellinger, 1972). Precooking meats may enhance the development of oxidative rancidity (Schamberger et al., 1977). Brotsky and Everson (1973) reported that use of sodium phosphates significantly reduced rancidity in cooked roast beef; this "technical effect" was recognized by USDA-FSIS in allowing the expanded use of phosphates in precooked meat products. The aim of the current research was to determine effects of reduced NaCl levels, reduced NaCl levels with the addition of sodium tripolyphosphate (STP) and reduced NaCl levels with the addition of Lem-0-Fos® (STP and a natural antioxidant) on properties of precooked pork sausage patties during extended periods of frozen storage.

MATERIALS & METHODS

A COMPANION PAPER (Matlock et al., 1984) describes the materials and methods utilized in a similar experiment involving raw-frozen pork sausage patties. This experiment with precooked-frozen pork sausage patties followed the same materials and methods (including the statistical analysis) with the exception of the precooking and reheating procedures, which are described below.

Cooking procedure

Patties were weighed and then deep fat fried in commercial pork lard at a cooking temperature of 165–168°C until a product temperature of 71–73°C was achieved (temperatures measured with thermocouples). These precooked patties were placed on wire racks to drain and cool to room temperature before reweighing to determine cooking yield. They were then chilled at –9°C to a frozen state before packaging and storage (–9°C) for 0, 2, 4 or 8 wk.

Reheating procedure

Before serving to panelists, precooked patties were reheated, from the frozen state, by placing them on stoneware plates covered with Saran wrap and heating them in a Litton Minute Master microwave oven for 90 sec on a temperature setting of "Reheat." A dwell time of 5 min after microwave reheating was allowed for temperature equilibration of the patties. Reheated product temperatures of approximately 60°C were achieved by using these methods. Weights before and after reheating by microwave were obtained to determine reheating yield.

RESULTS & DISCUSSION

MAIN EFFECT MEANS for properties of precooked-frozen sausage patties are presented in Table 1. Significant interaction means are presented in subsequent tables, as indicated in this discussion.

Moisture, fat and NaCl content

Moisture and fat content were not significantly affected by salt level or phosphate group. As expected, NaCl content was affected (P < 0.0001) by the level of NaCl added to the sausage formulation. The NaCl content of the patties was slightly higher than the level of NaCl added to the sausage formulation, indicating a concentration of NaCl due to shrinkage of the patties during precooking. Phosphate group did not affect (P > 0.05) the NaCl content.

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Table 1—Main effect means for properties of precooked-frozen sausage patties[a]

Treatments	Moisture (%)[b]	Fat (%)[b]	NaCl (%)[b]	Saltiness[c]	pH[b]	Cooking yield (%)[b]	Reheating yield (%)[c]	Juiciness[c]	Texture[c]	TBA number	Off-flavor[c]
Salt level											
1.5% NaCl	53.56[d]	28.03[d]	1.99[d]	5.9[d]	5.93[d]	70.2[d]	*	*	5.1[d]	*	6.2[d]
1.0% NaCl	53.76[d]	27.32[d]	1.47[e]	5.1[e]	5.94[d]	68.6[d]	*	*	5.2[d]	*	6.3[d]
0.5% NaCl	54.05[d]	27.43[d]	0.75[f]	4.0[f]	5.94[d]	68.9[d]	*	*	5.2[d]	*	6.4[d]
0.0% NaCl	52.90[d]	28.45[d]	0.27[g]	2.2[g]	5.87[d]	67.4[d]	*	*	5.4[d]	*	6.3[d]
Phosphate group											
No phosphate	52.96[d]	28.20[d]	1.10[d]	4.0[e]	5.73[e]	63.9[e]	95.6[d]	*	5.4[d]	*	6.0[e]
STP	54.05[d]	27.50[d]	1.13[d]	4.4[d]	5.98[d]	70.8[d]	95.2[d]	*	5.2[d]	*	6.5[d]
Lem-0-Fos®	53.71[d]	27.72[d]	1.13[d]	4.5[d]	6.04[d]	71.6[d]	94.7[d]	*	5.1[d]	*	6.4[d]
Storage (wk)											
0				4.1[e]				*	*	5.3[d]	*	6.9[d]
2				4.3[de]				*	*	5.2[d]	*	6.3[e]
4				4.5[d]				*	*	5.2[d]	*	6.1[e]
8				4.3[de]				*	*	5.2[d]	*	6.0[e]
Packaging											
Vacuum packaged				4.3[d]				95.2[d]	5.3[d]	5.2[d]	*	6.5[d]
PVC-wrapped				4.3[d]				95.1[d]	5.3[d]	5.3[d]	*	6.1[e]

[a] * indicates interaction occurred.
[b] n=9 for salt level, n=12 for phosphate group.
[c] Sensory scales were: saltiness, 8=extremely salty, 1=extremely not salty; juiciness, 8=extremely juicy, 1=extremely dry; texture, 8=extremely crumbly, 1=extremely not crumbly; off-flavor, 8=extremely weak to none, 1=extremely strong. n=72 for salt level and storage period, n=96 for phosphate group, n=144 for packaging.
[defg] Means within a column for each treatment followed by a common letter superscript are not different (P>0.05).

Saltiness

Higher NaCl levels resulted in significantly higher saltiness scores. Saltiness scores were also increased (P < 0.01) through the addition of phosphates. Although STP or Lem-0-Fos® did not significantly increase NaCl content, means for NaCl content of patties made with phosphates were numerically higher. The fact that panelists were able to detect such slight increases in NaCl content indicates that they are extremely sensitive to any changes in the NaCl content. Saltiness scores were also affected (P < 0.0001) by storage period. This may be due to declines in juiciness and reheating yield that also occurred in precooked patties during extended periods of frozen storage. Packaging method did not affect (P > 0.05) saltiness scores.

pH

pH values were not affected (P > 0.05) by NaCl level. Although NaCl is known to increase pH values, the precooking operation may have had the major effect on pH values in this study. Watts and Peng (1947) found no pH differences in cooked frozen ground pork with 0.0 or 1.5% NaCl added. The use of phosphates increased (P < 0.01) pH value. Alkaline phosphates are known to raise the pH of meat systems, thus increasing the water-holding capacity (WHC) of meat products, which results in greater juiciness and texture and improved cooking yields (Ellinger, 1972).

Cooking yield

Cooking yield was not affected (P > 0.05) by NaCl level, but the use of phosphates resulted in higher (P < 0.0001) cooking yields. These results establish a direct relationship between higher pH values and increased cooking yields. Sofos (1983) found a similar relationship between pH values and cooking yield for beef/pork frankfurters.

Reheating yield

Reheating yields were not affected (P > 0.05) by phosphate group. This result suggests a contradiction to previous findings in this study which show an enormous benefit for yield values through the addition of phosphates. However, a calculation of total yield (cooking yield x reheating yield) reveals that there was a great advantage in using STP or Lem-0-Fos® for precooked patties. Means for total yield were 61.1%, 67.4% and 67.8% for patties containing no phosphate, STP and Lem-0-Fos®, respectively. Reheating

Table 2—Significant interaction means for juiciness scores and TBA number stratified by phosphate group and salt level

	Juiciness[a]	TBA number[b]
No phosphate		
1.5% NaCl	5.0[d]	1.35[d]
1.0% NaCl	4.8[e]	1.78[c]
0.5% NaCl	4.4[f]	0.78[e]
0.0% NaCl	3.7[g]	0.64[ef]
STP		
1.5% NaCl	6.1[c]	0.54[fg]
1.0% NaCl	6.1[c]	0.61[ef]
0.5% NaCl	5.4[d]	0.45[fgh]
0.0% NaCl	4.7[ef]	0.25[h]
Lem-0-Fos®		
1.5% NaCl	6.2[c]	0.47[fgh]
1.0% NaCl	6.0[c]	0.40[fgh]
0.5% NaCl	5.5[d]	0.40[fgh]
0.0% NaCl	5.0[e]	0.32[gh]

[a] 8=extremely juicy, 1=extremely dry. n=24.
[b] mg malonaldehyde/kg. n=12.
[cdefgh] Means within a column followed by a common letter superscript are not different (P>0.05).

yields were not affected by the phosphate treatment because the majority of the shrinkage in precooked patties takes place when they are precooked (cooking yield). Significant interaction means for reheating yield stratified by storage period and salt level are shown in Table 3. Within storage periods, patties at the 0 wk were not different (P > 0.05), regardless of salt level. However, at 4 and 8 wk storage periods, patties made with 0.0 or 0.5% NaCl tended to have significantly lower reheating yields than patties made with 1.0 or 1.5% NaCl. Within salt levels, patties made with 1.5 or 1.0% NaCl were not significantly lower in reheating yield until the 8 wk storage period. However, patties made with 0.5 or 1.0% NaCl were significantly lower in reheating yield at the 4 wk storage period. This interaction indicates that a minimum level of 1.0% NaCl is necessary to maintain reheating yields of precooked patties during extended periods of frozen storage. The relationship between higher NaCl levels and improved yield, texture and sensory properties has been reviewed by Terrell (1983). Packaging method did not affect (P > 0.05) reheating yields.

Juiciness

Significant interaction means for juiciness scores stratified by phosphate treatment and salt level are shown in

Table 2. Within phosphate treatment, higher NaCl levels resulted in higher juiciness scores. At each NaCl level, the use of phosphates significantly improved juiciness scores over those scores for patties made without phosphates. Significant interaction means for juiciness scores stratified by storage period and salt level are shown in Table 3. Within storage periods, patties made with 1.0% NaCl were not different (P > 0.05) in juiciness from patties made with 1.5% NaCl until the 8 wk storage period. Patties made with 0.0 or 0.5% NaCl were lower (P < 0.05) in juiciness than patties made with 1.5% NaCl at all storage periods. Within salt levels, patties made with 1.0 or 1.5% NaCl did not decrease (P > 0.05) in juiciness throughout all storage periods. Patties made with 0.0 or 0.5% NaCl had significant declines in juiciness as they were held for extended periods of frozen storage. This interaction reveals that a minimum level of 1.0% NaCl is necessary to maintain juiciness scores of precooked patties during extended periods of frozen storage. Packaging method did not affect (P > 0.05) juiciness scores (Table 1).

Table 3—Significant interaction means for reheating yield, juiciness scores and TBA number stratified by storage period and salt level

	Reheating yield (%)[a]	Juiciness[b]	TBA no.[c]
0 wk			
1.5% NaCl	97.2[d]	5.9[d]	0.48[gh]
1.0% NaCl	96.9[de]	5.7[de]	0.50[fgh]
0.5% NaCl	96.9[de]	5.4[efg]	0.30[h]
0.0% NaCl	96.2[de]	4.8[h]	0.27[h]
2 wk			
1.5% NaCl	97.3[d]	6.0[d]	
1.0% NaCl	97.0[de]	5.6[def]	
0.5% NaCl	96.7[de]	5.2[fgh]	
0.0% NaCl	95.7[ef]	4.8[h]	
4 wk			
1.5% NaCl	96.4[de]	6.0[d]	0.59[efg]
1.0% NaCl	96.1[de]	5.8[de]	0.77[ef]
0.5% NaCl	92.2[g]	5.0[gh]	0.44[gh]
0.0% NaCl	91.4[g]	4.3[i]	0.35[gh]
8 wk			
1.5% NaCl	95.7[ef]	6.0[d]	1.29[d]
1.0% NaCl	94.5[f]	5.4[efg]	1.53[d]
0.5% NaCl	92.0[g]	4.8[h]	0.86[e]
0.0% NaCl	90.5[h]	3.9[i]	0.58[fg]

[a] n=18.
[b] 8=extremely juicy, 1=extremely dry. n=18.
[c] mg malonaldehyde/kg. n=12.
[defg] Means within a column followed by a common letter superscript are not different (P>0.05).

Table 4—Significant interaction means for TBA number stratified by storage period and phosphate group

	TBA number[a]
0 wk	
No phosphate	0.68[d]
STP	0.24[e]
Lem-0-Fos®	0.23[e]
4 wk	
No phosphate	1.04[c]
STP	0.29[e]
Lem-0-Fos®	0.29[e]
8 wk	
No phosphate	1.70[b]
STP	0.86[cd]
Lem-0-Fos®	0.64[d]

[a] mg malonaldehyde/kg. n=16.
[bcde] Means within a column followed by a common letter superscript are not different (P>0.05).

Texture

Texture scores were not affected (P > 0.05) by salt level, phosphate group, storage period or packaging. Patties made with higher NaCl levels or with phosphates had numerically lower texture scores, indicating more bind for these patties. It appears that the effect of the precooking process masked any real differences in texture that might have occurred due to NaCl/phosphate combinations.

TBA number

Significant interaction means for TBA number stratified by phosphate group and salt level are shown in Table 2. Within each phosphate group, an increase in NaCl level tended to increase TBA number, especially in patties containing no phosphate. At each salt level, patties containing no phosphate had significantly higher TBA values than patties made with STP or Lem-0-Fos®. This interaction indicates that in the absence of alkaline phosphates, higher NaCl levels will result in higher TBA values. The use of phosphates will lower the TBA number, regardless of NaCl level. These results support the findings of Judge and Aberle (1980) and Keeton (1983). Significant interaction means for TBA number stratified by storage period and salt level are shown in Table 3. Within storage periods, patties at the 0 wk were not different (P > 0.05) in TBA number, regardless of NaCl level. As storage time increased, patties made with higher NaCl levels tended to have higher TBA values. At all NaCl levels, patties at 4 wk of storage were not significantly higher in TBA number than at 0 wk of storage. At the 8 wk storage period, all patties were higher (P < 0.05) in TBA than at 4 wk of storage except for patties made with 0.0% NaCl. This interaction indicates that NaCl has a prooxidant effect on precooked sausage patties during extended periods of frozen storage. Rhee et al. (1983) found that a similar prooxidant effect occurred in ground salted pork samples stored at 5°C. Significant interaction means for TBA number stratified by storage period and phosphate group are shown in Table 4. At all storage periods, the use of phosphates significantly improved TBA values over those for patties containing no phosphate. Within phosphate groups, patties containing no phosphate were significantly higher in TBA number at each successive storage period. However, patties made with STP or Lem-0-Fos® were not significantly higher in TBA number until the 8 wk storage period. This interaction again demonstrates the strong antioxidant effect obtained with the use of alkaline phosphates in precooked sausage patties. Significant interaction means for TBA number stratified by storage period and packaging are shown in Table 5. None of the TBA values was different (P > 0.05) except for those patties stored for 8 wks in PVC. This interaction indicates that vacuum packaging is an effective method for controlling rancidity in precooked sausage patties over extended periods of frozen storage.

Table 5—Significant interaction means for TBA number stratified by storage period and packaging

	TBA number[a]
0 wk	
Vacuum packaged	0.39[b]
PVC-wrapped	0.39[b]
4 wk	
Vacuum packaged	0.51[b]
PVC-wrapped	0.56[b]
8 wk	
Vacuum packaged	0.58[b]
PVC-wrapped	1.55[c]

[a] mg malonaldehyde/kg. n=24.
[bc] Means within a column followed by a common letter superscript are not different (P>0.05).

Off-flavor

Off-flavor scores were not affected (P > 0.05) by salt level, but the addition of phosphates affected (P < 0.01) these scores. Patties made with STP or Lem-0-Fos® had significantly higher off-flavor scores (less off-flavor) than patties containing no phosphate. These results indicate that the antioxidant effect of alkaline phosphates is more potent than the prooxidant effect of NaCl in precooked sausage patties. Off-flavor scores were also affected (P < 0.0001) by storage period. Off-flavor scores at 2, 4 and 8 wk of storage were not different (P > 0.05), but were significantly lower (more off-flavor) than off-flavor scores at the 0 wk storage period. Reagan et al. (1983) also found that extended storage periods significantly reduced flavor acceptance ratings for fresh pork sausage patties. Packaging also affected (P < 0.01) off-flavor scores, as the use of vacuum packaging significantly improved off-flavor scores over those for PVC-wrapped patties.

CONCLUSIONS

THE RESULTS of this study indicate that the addition of alkaline phosphates to precooked patties would increase pH values, cooking yield, saltiness and juiciness scores while protecting against the formation of off-flavor and rancidity. A reduction in NaCl content without a loss of product quality and flavor would be feasible if phosphates were added to compensate for any loss of product functionality that might occur when NaCl levels are reduced. A minimum level of 1.0% NaCl in precooked sausage patties would be desirable to maintain yield and sensory properties over extended periods of frozen storage.

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Qualities of Chunked and Formed Lamb Roasts

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──────── ABSTRACT ────────

Influence of mechanically separated lamb (MSL), NaCl level, processing conditions and extenders on composition and quality of 72 chunked and formed lamb roasts were evaluated. Roasts with 0.5% NaCl had significantly (P<0.05) lower juiciness and flavor scores than roasts containing 1.0-2.0% NaCl. Muscle chunk separation was more extensive and Instron breaking strength values were lower at the 0.5% NaCl level. Roasts containing 30% MSL were more tender and juicy than roasts containing 10% MSL and they had less cook loss. Soy-extended roasts had less desirable juiciness. flavor and texture scores and were lower in fat and lighter in color than control roasts or roasts containing dried skim milk. All roast formulations had TBA values below 1.0.

INTRODUCTION

BETWEEN 1966 AND 1980, U.S. per capita lamb consumption dropped 69% and lamb production fell 48% while the average price rose 22% USDC, 1980). According to the Economic Research Service (1977), the longterm downward trend in per capita lamb consumption is due to shrinking supplies and rising retail costs. Lamb production has been directed towards the sale of fresh cuts to individual households. Since the retail lamb market is not growing, producers and processors may need to (1) look to new market areas, and (2) look to new product development technologies which can increase consumer acceptance and improve the unit price, making lamb more competitive with other red meat. Growth of volume sales to institutional concerns has inspired the meat industry to engineer standardized, portion-controlled beef and pork products for these markets. Lamb, like beef and pork, may find a valuable new market in this area if current processing technologies can be applied.

Restructured roasts and chops are engineered products which offer many of the characteristics desired by large-volume food handlers.

Nonmeat extenders such as soy protein and dried skim milk solids offer a variety of processing and functional characteristics in processed meat products as well as cost-reduction alternatives to all-meat items (Moore et al., 1976). Mechanical separation increases the yield of meat per carcass and reduces labor costs compared to hand boning (Field, 1981). Mechanically separated lamb (MSL) and nonmeat extenders in conjunction with restructuring technology may be useful in production of intermediate value lamb products.

The purpose of this study was to determine the effects of salt level, mechanically separated lamb (MSL) level, extender and processing treatment on sensory and binding characteristics of chunked and formed lamb roasts.

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Methods & Materials

Roast formulation

Ninety-nine ram and wether lambs weighing approximately 46 kg each were slaughtered in four groups of 24 or 25 lambs per group at the Roman L. Hruska Meat Animal Research Center. Lambs were hot boned 2 hr postmortem. Boneless meat and skeletons were wrapped separately, chilled overnight at 4°C and transported to the Univ. of Wyoming within 24 hr of slaughter.

Boneless meat was hand-trimmed to remove visible fat, then lean combined from the leg, loin, rib and shoulder of 25 lambs was ground through a 3.81 cm kidney plate. Lamb skeletons were ground through the 1.27 cm holes of a Wiler grinder (model BA-8). Meat was separated from skeletons using a Beehive Deboner (model AUX-709) with 0.46 mm holes in the sieve. MSL yields were approximately 50%.

Four batches of roasts were prepared to contain 2.0%, 1.5%, 1.0% or 0.5% NaCl. For each salt level, chilled lean and MSL were weighed then mixed separately for 10 min in a Leland Double Action food mixer (model 200 DA) with the appropriate concentration of NaCl and 0.3% sodium tripolyphosphate (FOS-6, Griffith's Labs). Because both fresh and cured roasts were prepared, 156 ppm nitrite (Modern Cure, Heller's Labs) was added to 20 kg of lean and 20 kg MSL removed from each NaCl level. Concentrations of additives were based on 100% lean or MSL. Lean and MSL mixtures were held at 4°C overnight. Within each salt level, roasts with and without nitrite were formulated to contain either 10% or 30% of the lean portion as MSL. Within each MSL level, nitrite level, and NaCl level, control roasts (no extender), roasts extended with 3.5% calcium-reduced dried skim milk (Savortex, Western Dairies, San Francisco, CA) and roasts extended with 3.5% soy isolate plus 17.3% rehydrated structured soy protein (SPF-200 and Supro-620, Ralston Purina, St. Louis, MO) were prepared. Appropriate amounts of lean, MSL and extender were combined in 15 and 20 kg batches and mixed for 5 min in a Leland food mixer. Additional NaCl, phosphate and nitrite were included in extended roasts to compensate for extender dilution.

Roast mixtures were stuffed into 100 mm fibrous casings using a Koch stuffer (model KF-100) and held at 4°C overnight. Roasts prepared without nitrite were either frozen to −30°C for 3 days and then heated to an internal temperature of 71°C from the frozen state, or preheated from the chilled state to 63°C, chilled at 4°C for 3 days, then reheated to 71°C. For heat processing, roasts were hung on racks in a smokehouse and heated without smoke according to the following schedule: 60°C for 2 hr, 71°C for 2 hr, and 85°C to desired internal temperature. Roasts were showered with cold water to bring internal temperature to 90°C. Once processed to 71°C internal temperature and showered to 90°C, all roasts were chilled at 4°C overnight prior to collection of sensory data.

Sensory evaluation

Fifty to 75-member consumer-type panels evaluated chilled lamb roasts for tenderness, juiciness and desirability using an 8-point scale (1 = extremely tough, dry or undesirable and 8 = extremely tender, juicy or desirable). At each panel session, panelists chose samples in random order from four different lamb roasts which were sliced 0.64 cm thick, cut into eight pie-shaped wedges, coded and served on white paper plates. At each session, panelists were told that they were to evaluate a "processed meat item" and analyses included only responses from panelists who evaluated all four samples within a session. Within each group of four samples served at a session, variables included level of MSL (10% and 30%) and use of extender (control to DSM, control to soy, and DSM to soy). Level of salt, presence or absence of nitrite, and type of heat treatment (frozen and heated or preheated, chilled and reheated) were con-

stant within a group of four samples evaluated at one session and each treatment was replicated four times. Drinking water was provided for rinsing between samples.

Appearance scoring

Roasts heated to 71°C then chilled overnight were scored for color and muscle chunk separation. A 2.54 cm thick slice was cut from the center of each roast, coded and placed on white freezer paper under fluorescent light for evaluation. Five experienced meat lab personnel scored 18 roast formulations in four groups corresponding to the four salt levels on a 5-point scale where 1 = light red color or no evidence of muscle chunk separation and 5 = dark red color or extensive muscle chunk separation.

Bind evaluation

Bind is defined as the tendency for the MSL and meat chunks of a restructured roast slice to adhere. Bind was evaluated by measuring breaking strength, elongation and total energy required to break through a slice of roast.

Heated chilled roast slices cut 1.27 cm thick were pressed onto 1 mm needles mounted 2 mm apart around the perimeter of a plexiglass ring 73 mm in diameter. A 2.54 cm steel ball mounted on the crosshead of an Instron Universal Testing maching moving at a rate of 10 cm/min was pressed through the roast slice. Breaking strength is the peak load in kg necessary for the ball to burst through a slice of restructured roast. Elongation is the distance in mm traveled by the ball after contact with the roast slice before the slice broke (Field et al., 1983). Total energy was calculated by converting the area under the breaking strength-elongation peak to joules (Instron Corporation Manual, 1967). The average of three measurements on each roast was used for statistical analysis.

Chemical characteristics

Fat, moisture, protein and ash content were determined on fresh lean and MSL and on heated and chilled roasts using standard AOAC methods (1980).

Thiobarbituric acid values were determined on all roasts heated to 71°C and chilled to 4°C using the methods described by Sinnhuber and Yu (1977).

Thirty grams of raw lean or MSL were slurried with 100 mL water and pH readings were taken using a Fisher Accumet pH meter (model 600).

Total pigment content of lean and MSL constituents of the roasts was determined as outlined by Franke (1973).

Statistical analysis

Least-squares means for differences in palatability, appearance, objective measurements and composition due to NaCl level, MSL level, extender and processing treatment were determined as outlined by Harvey (1975). Differences between means were tested using Duncan's Multiple Range Test (Steele and Torrie, 1960).

RESULTS & DISCUSSION

THE CHARACTERISTICS of lean and MSL for formulation of restructured lamb roasts are presented in Table 1. Mechanically separated lamb was higher in total pigment, pH, fat, ash and calcium and lower in protein and moisture than lamb lean.

The effects of NaCl level on characteristics of restructured lamb roasts are presented in Table 2. Salt level had no significant effect on either tenderness or texture. Roasts containing 0.5% NaCl were less juicy and had less desirable flavor than roasts containing 1.0-2.0% NaCl. As level of NaCl increased, scores for muscle chunk separation decreased (P<0.05).

Although total energy and elongation were unaffected, breaking strength increased (P<0.05) as NaCl level increased from 0.5% to 2.0% indicating that salt level increased binding strength in the roast slice. Binding of meat chunks due to myosin extraction and heat-induced gelation is facilitated by these salt levels (Siegel and Schmidt, 1979; Booren et al., 1982). Salt level had no significant effect on percentage cook loss although compositional differences in cooked products indicated that fat and moisture stabiliza-

Table 1—Characteristics of lean and mechanically separated lamb used in restructured lamb roasts

Variable	Component	
	Lean	MSL
Total pigment, mg/g	3.65	5.13
pH at 24 hr postmortem	5.7	6.5
Composition		
Moisture, %	71.76	58.57
Fat, %	7.94	22.59
Protein, %	18.44	15.02
Ash, %	1.02	1.76
Calcium, %	0.01	0.38

Table 2—Least-squares means for characteristics of restructured lamb roasts containing varying levels of NaCl[a]

	NaCl level				
	2.0%	1.5%	1.0%	0.5%	S.E.
Sensory characteristics[b]					
Tenderness	5.86	5.79	5.85	5.84	0.06
Juiciness	5.38[j]	5.48[j]	5.39[j]	5.17[i]	0.06
Flavor	5.30[j]	5.46[j]	5.34[j]	5.12[i]	0.05
Texture	5.25	5.43	5.27	5.21	0.05
Appearance					
Muscle chunk sepn[c]	2.39[j]	2.71[i,j]	2.72[i,j]	3.11[i]	0.12
Color[d]	2.72[i]	3.10[j]	2.95[i,j]	3.06[j]	0.09
Instron measurements					
Breaking strength, kg[e]	2.89[i]	2.53[k]	2.03[l]	2.68[j]	0.12
Total energy, J[f]	0.37	0.37	0.38	0.39	0.05
Elongation, mm[g]	49.22	55.62	58.89	42.30	7.11
Cook loss, %	12.91	12.39	12.14	11.07	0.69
TBA[h]	0.55[k]	0.41[i]	0.68[l]	0.44[j]	0.04
Composition					
Moisture, %	65.75[j]	66.98[i]	66.35[i,j]	66.88[i]	0.24
Fat, %	11.23[i]	9.85[k]	10.46[j]	10.52[j]	0.18
Protein, %	18.43	18.72	18.89	19.80	0.17
Ash, %	3.34[k]	2.96[j]	2.26[i]	1.98[l]	0.06

[a] Includes roasts heated to 63°C, chilled and reheated with and without nitrite as well as roasts which were frozen and heated to 71°C. (N=72)
[b] 1 = extremely tough, dry or undesirable, 8 = extremely tender, juicy or desirable.
[c] 1 = no muscle chunk separation, 5 = extensive muscle chunk separation.
[d] 1 = light red, 5 = dark red.
[e] Peak force in kg required to break through a 1.27 cm roast slice.
[f] Integrated area under the break strength, elongation curve converted to joules.
[g] Distance which sample extended before breaking after ball contacted slice surface.
[h] Mg malonaldehyde/1000g cooked sample.
[i,j,k,l] Superscripted means in the same row are significantly different (P < 0.05).

tion were affected. As NaCl level increased over 1.5%, moisture content decreased while fat content increased; this may reflect a peak in the tendency for NaCl to increase water-holding capacity which occurs between 1.5% and 2.0% NaCl along with a concommitant change in fat content. Differences in TBA values followed no consistent pattern with respect to NaCl level, and values below 1.0 indicate no significant rancidity problem.

The effects of MSL level on palatability, appearance, objective measurements and composition are presented in Table 3. Roasts containing 30% MSL were more tender and Juicy (P<0.05) than roasts containing 10% MSL. Increased tenderness due to fine comminution of a larger proportion of the product has been reported by Cross et al. (1977, 1978b) and Breidenstein (1982). Reduction in the amount of connective tissue in mechanically separated meat probably makes a minor contribution to tenderness in this system. Increased juiciness in roasts containing 30% MSL is

Table 3—Least-squares means for characteristics of restructured lamb roasts containing 10% or 30% MSL[a]

Variable	Level of MSL		
	10%	30%	S.E.
Sensory characteristics[b]			
Tenderness	5.70[j]	5.96[i]	0.04
Juiciness	5.28[j]	5.42[i]	0.04
Flavor	5.31	5.30	0.04
Texture	5.28	5.33	0.04
Appearance			
Muscle chunk sepn[c]	2.80	2.66	0.09
Color[d]	3.08[j]	2.84[i]	0.06
Instron measurements			
Breaking strength, kg[e]	2.60	2.47	0.07
Total energy, J[f]	0.34[j]	0.41[i]	0.03
Elongation, mm[g]	53.15	53.36	4.33
Cook loss, %	12.93[i]	11.33[j]	0.48
TBA,[h]	0.47	0.51	0.03
Composition			
Moisture, %	66.75[i]	66.23[j]	0.17
Fat, %	10.00[j]	11.04[i]	0.13
Protein, %	19.14	18.78	0.12
Ash, %	2.58	2.70	0.04

[a] Includes roasts heated to 63°C, chilled and reheated to 71°C as well as roasts heated to 71°C from the frozen state.
[b] 1 = extremely tough, dry or undesirable, 8 = extremely tender, juicy or desirable.
[c] 1 = no muscle chunk separation, 5 = extensive muscle chunk separation.
[d] 1 = light red, 5 = dark red.
[e] Peak force in kg required to break through a 1.27 cm roast slice.
[f] Integrated area under the break strength-elongation curve converted to joules.
[g] Distance which sample extended before breaking after ball contacted slice surface.
[h] Mg malonaldehyde/1000g cooked sample.
[i,j] Superscripted means in the same row are significantly different (P < 0.05).

Table 4—Least-squares means for characteristics of frozen, cooked and cured restructured lamb roasts

Variable	Processing treatment			
	Frozen	Heated	Cured	S.E.
Sensory characteristics[a]				
Tenderness	5.81	5.86	5.82	0.05
Juiciness	5.43	5.31	5.31	0.05
Flavor	5.31	5.25	5.36	0.04
Texture	5.38	5.26	5.28	0.04
Appearance				
Muscle chunk sepn[b]	2.67[i]	3.04[h]	2.49[i]	0.11
Color[c]	2.73[i]	2.98[h]	3.17[h]	0.08
Instron measurements				
Breaking strength, kg[d]	2.36	2.56	2.69	0.09
Total energy, J[e]	0.39[h]	0.43[h]	0.30[i]	0.04
Elongation, mm[f]	39.60	40.85	39.65	0.70
Cook loss, %	9.45[i]	13.58[h]	13.35[h]	0.59
TBA[g]	0.61[h]	0.47[i]	0.41[i]	0.03
Composition				
Moisture, %	67.37[h]	66.10[i]	66.00[i]	0.21
Fat, %	10.56	10.62	10.38	0.16
Protein, %	18.15[i]	19.36[h]	19.37[h]	0.15
Ash, %	2.63	2.59	2.70	0.05

[a] 1 = extremely tough, dry or undesirable, 8 = extremely tender, juicy or desirable.
[b] 1 = no muscle chunk separation, 5 = extensive muscle chunk separation.
[c] 1 = light red, 5 = dark red.
[d] Peak force in kg required to break through a 1.27 cm roast slice.
[e] Integrated area under the break strength-elongation curve converted to joules.
[f] Distance which sample extended before breaking after ball contacted slice surface.
[g] Mg malonaldehyde/1000g cooked sample.
[h,i] Superscripted means in the same row are significantly different (P < 0.05).

in agreement with Gillett et al. (1976), Field et al. (1977) and Cross et al. (1977, 1978a, b). Roasts containing 30% MSL contained 1% more fat in the finished product than roasts containing 10% MSL. The calculated correlation coefficient between fat content and juiciness was +0.49, which was statistically significant at the P<0.05 level. Higher moisture and lower fat content of roasts prepared with 10% MSL compared to those with 30% MSL reflects the compositional differences of the original components (Tables 1 and 3). Roasts containing 30% MSL were lighter red than roasts containing 10% MSL. Although total pigment content of MSL was greater, increased fat introduced into the product with MSL may have negated the effect of pigment concentration. Level of MSL had no effect on muscle chunk separation score, breaking strength or elongation, while more total energy was required to break through roast slices containing 30% MSL. These data indicate that 30% MSL produced a product which was as strongly bound as one containing 10% MSL. The removal of heavy connective tissue from MSL may allow for more uniform binding of muscle proteins (Field, 1976). Roasts containing 30% MSL lost less weight (P<0.05) during heat processing than did roasts containing 10% MSL. Level of MSL had no effect on TBA values.

The effects of heat processing treatment on roast characteristics are presented in Table 4. Palatability was unaffected by processing treatment. Roasts which were preheated in the presence or absence of nitrite had darker red color scores (P<0.05) than roasts heated once from the frozen state. Neither breaking strength nor elongation was affected by heat processing treatment. Cured roasts exhibited muscle chunk separation scores similar to roasts heated from the frozen state, while cured roasts required less total energy to break. More muscle chunk separation occurred in roasts preheated from the fresh state than in either of the other two treatments, but these roasts required the same amount of total energy to break. The relationship between nitrite and bind is unclear. Roasts heated twice lost more weight, were lower in moisture and higher in fat than roasts heated once from the frozen state. Roasts heated from the frozen state had slightly higher TBA values than roasts which were preheated, chilled and reheated.

The effects of extenders on restructured roast characteristics are presented in Table 5. While extenders had little effect on tenderness, soy extender lowered juiciness, flavor and texture desirability scores (P<0.05) when compared to DSM and control roasts. Roasts containing DSM exhibited the most desirable flavor scores of the three extender treatments. Soy-extended roasts contained approximately 2% less fat than DSM-extended roasts and 3% less fat than control roasts. Because TBA values for soy-extended roasts were not higher than for control products, flavor differences were not attributable to oxidative rancidity. Hand and Crenwelge (1981) and The Committee on Animal Products (1982) have suggested that flavor reversion of soy components may be responsible for less acceptable flavor in soy-extended meat products. Soy-extended roasts had less desirable texture than either of the other two treatments. When the three extender treatments were compared, muscle chunk separation was least evident in soy-extended roasts. Additionally, no differences in bind strength existed among the three treatments when Instron values were compared. Lighter red color of soy-extended roasts in this study is attributed to dilution of meat pigments by light colored soy isolate, an effect which has been cited by Judge et al. (1974) and Seideman et al. (1979). Although soy- and DSM-extended roasts had lower TBA values than control roasts, all TBA values were below 1.0 and were considered

Table 5—Least-squares means for characteristics of lamb roasts containing no extender, dried skim milk solids or soy isolate[a]

Variable	Extender			S.E.
	Control	Dried milk	Soy isolate	
Sensory characteristics[b]				
Tenderness	5.91	5.85	5.73	0.05
Juiciness	5.53[i]	5.54[i]	4.99[j]	0.05
Flavor	5.37[j]	5.52[i]	5.03[k]	0.04
Texture	5.43[i]	5.40[i]	5.08[j]	0.04
Appearance				
Muscle chunk sepn[c]	2.70[j]	3.01[i]	2.48[k]	0.11
Color[d]	3.55[i]	3.41[i]	1.92[j]	0.07
Instron measurements				
Breaking strength, kg[e]	2.59	2.56	2.47	
Total energy, J[f]	0.40	0.39	0.35	0.04
Elongation, mm[g]	40.28	40.84	39.00	0.68
Cook loss, %	12.16	11.09	12.33	0.59
TBA[h]	0.59	0.46[j]	0.47[j]	
Composition				
Moisture, %	65.79[j]	66.66[i]	67.02[i]	0.21
Fat, %	11.76[i]	10.83[j]	8.96[k]	0.16
Protein, %	18.24[j]	17.91[k]	20.56[i]	0.15
Ash, %	2.62	2.69	02.61	0.05

[a] Includes roasts heated to 63°C, chilled and reheated as well as roasts which were frozen and heated to 71°C. (N = 72).
[b] 1 = extremely tough, dry or undesirable, 8 = extremely tender, juicy or desirable.
[c] 1 = no muscle chunk separation, 5 = extensive muscle chunk separation.
[d] 1 = light red, 5 = dark red.
[e] Peak force in kg required to break through a 1.27 cm roast slice.
[f] Integrated area under the break strength-elongation curve converted to joules.
[g] Distance which sample extended before breaking after ball contacted slice surface.
[h] Mg malonaldehyde/1000g cooked sample.
[i,j,k] Superscripted means in the same row are significantly different (P < 0.05).

to be of little significance with respect to rancid flavor. Roasts containing soy protein were higher in moisture and protein and lower in fat than either DSM-extended or control roasts. Lower fat content reflects dilution of meat fat with soy isolate as fat content per unit meat was approximately the same for soy-extended and control roasts.

CONCLUSION

CHUNKED AND FORMED lamb roasts containing 1.0-2.0% NaCl were more juicy and had more acceptable flavor scores than roasts containing 0.5% NaCl. As salt level increased, breaking strength increased while muscle chunk separation decreased. Because addition of NaCl over 1.0% did not result in a significant increase in juiciness, flavor, muscle chunk separation, or cook loss, and because increasing salt level appeared to have detrimental effects on roast color and TBA values, it is suggested that salt levels of 1.0-1.5% may successfully be used in restructured lamb roasts. Incorporation of 30% MSL into restructured roasts increased tenderness, juiciness and fat while decreasing cook loss and moisture when compared to incorporation of 10% MSL. The higher level of MSL appeared to have no detrimental effect on product bind, so that 30% MSL in a restructured product is recommended due to its positive effects on palatability and yield. Sensory characteristics were unaffected by heat processing treatment, although

preheating had unpredictable effects on muscle chunk separation and product bind strength. Heating from the frozen state appears to be the most beneficial method of heat processing for chunked and formed lamb roasts since cook loss was significantly greater in preheated roasts and because preheating resulted in no positive changes. Soy extender reduced palatability, color darkness, fat content and muscle chunk separation when compared to control roasts. DSM extender increased flavor acceptability, muscle chunk separation and moisture content when compared to control roasts. While neither extender appeared to affect bind strength, control roasts or DSM-extended roasts were most palatable. Because all TBA values were below 1.0, oxidative rancidity was not considered to be a problem in roasts held for 3-5 days. However, differences in rancidity would be expected to be greater if roasts were held for longer periods of time.

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Component Interactions in the Extrusion Cooking Process: Influence of Process Conditions on the Functional Viscosity of the Wheat Flour System

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─────── ABSTRACT ───────

Wheat starch, vital gluten and wheat flour solubles were processed in combination, in a single screw extruder. The paste viscosity profile and degree of cook of the starch component were used to assess interactive effects. The composite of all 3 components, simulating a wheat flour, was more cooked and gave thicker pastes than wheat flour itself when processed under the same extrusion conditions. The data suggest that the integral structure of wheat flour particles imparts a greater resistance to water penetration and cooking than when the individual components exist at random in a composite formulation.

INTRODUCTION

THE EXTRUSION COOKING PROCESS represents one of the fastest growing process technologies in recent years. Using a variety of raw materials, products such as modified cereal flours and starches, textured proteins, snack foods, breakfast cereals and pet foods may be produced (Harper, 1981). In spite of the developments in process and extruder design serving this multi-billion dollar industry, there is a lack of published data in support of understanding both the nature of the process and the interactions occurring among components of an extruded formulation. Anderson et al. (1969) and Conway (1971) were among the first to describe process conditions in relation to product behavior. In particular, these authors found for corn grits that as screwspeed increased and moisture content decreased, the expansion index and water solubility increased while water absorption decreased.

Although several recent studies have been published on the physicochemical properties of extruded starches (Mercier and Feillet, 1975; Mercier et al., 1979; Colonna and Mercier, 1982) very little information has been published on the wheat flour system. Paton and Spratt (1978) showed that the functional viscosity of extruded chlorinated soft wheat flour was similar to that of extruded wheat starch. Unchlorinated wheat flour was more difficult to cook under the same extrusion conditions. This work pointed to the role of chlorine in facilitating water penetration into the flour starch granules thereby improving cooking. Kim and Rottier (1980) described extrusion studies of wheat flour at 170°C and 35% moisture that produced a product which could be substituted for chlorinated cake flour in bakery goods. Recently, Faubion and Hoseney (1982) examined the influence of various levels of gluten and lipids on the extrusion of wheat starch. They showed that the expansion index decreased as the gluten content increased. The addition of lipids had only a minor influence on extrudate expansion.

Very little information has been published on the relationships between extrusion conditions, component interactions and their
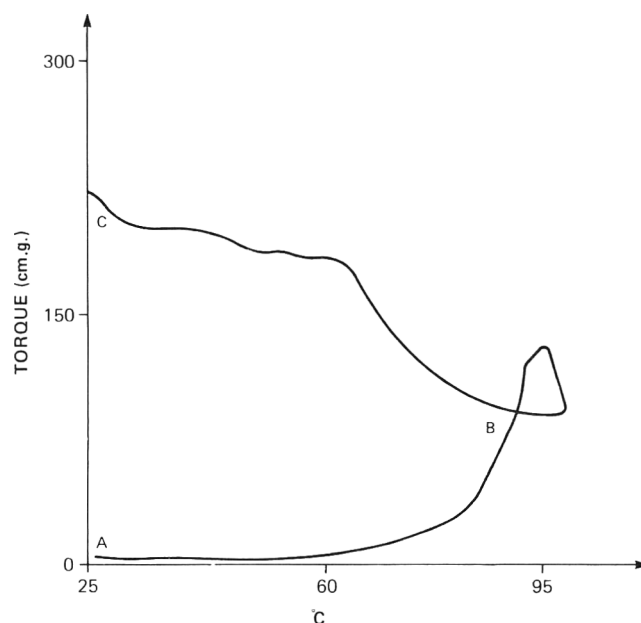

Fig. 1—Typical pasting curve of 9% (db) solids wheat starch, pH 6.8: (A) initial starting value; (B) intersection of heating and cooling curves; (C) final value; ABC, loop area.

effects on the consistency (viscosity) of the extrudates. The present study begins to address this subject using the wheat flour system, due to the commercial ease of obtaining the various component fractions from a parent flour. The objective was to determine, as a result of the extrusion process, the effects of gluten and water solubles on the pasting behavior of the wheat starch component and to compare these results to the behavior of wheat flour extruded under identical conditions.

MATERIALS & METHODS

WHEAT STARCH, vital gluten, wheat flour solubles and soft wheat flour (9.7% protein) were obtained courtesy of Ogilvie Flour Mills, Montreal, Quebec, Canada. Extrusions were conducted with a modified Brabender Model 2002 laboratory plastics extruder (L/D = 20:1; die size = 6.25 mm) as previously described by Paton and Spratt (1978).

Eight different combinations of screw speed, metering zone and die zone temperature were applied to each of the four feed materials under four different combinations of screw geometry and feed moisture content (Table 1). Thus 128 individual extrusions were performed in this study. The experimental design was not optimized due to the number of variables examined; nor was it the intention to obtain a response surface. It was more of interest to us to examine extrusion conditions where we knew that the functional behavior of starch was substantially or minimally altered and where these same conditions could be applied to the three other feed materials to determine the ease of cooking of the starch component in each case.

Composite flours were prepared to approximate the amount of vital gluten (VG) 10%, and wheat flour solubles (WFS) (5%) in soft wheat flour. Materials were dry blended in a twin shell "V" type mixer and preconditioned overnight to a final moisture content of 20.0±0.2 or 27±0.3%, respectively.

Materials were fed to the screw by means of a rotary paddle feeder and

Table 1—Extruder operating conditions

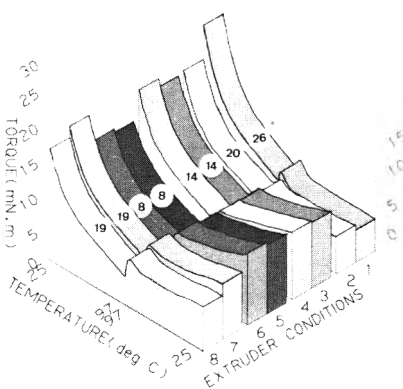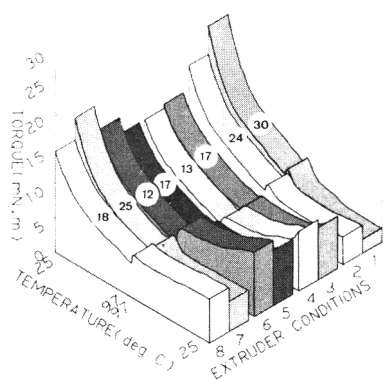| Code	rpm	Metering zone (°C)	Die (°C)
1	50	120	120
2	100	120	120
3	50	163	120
4	100	163	120
5	50	163	163
6	100	163	163
7	50	120	163
8	100	120	163

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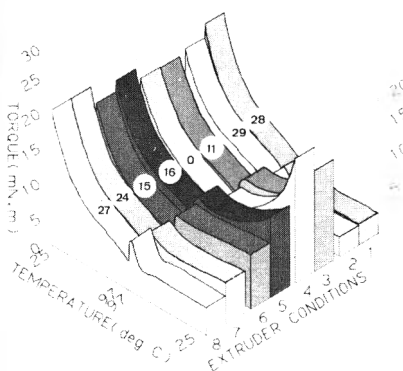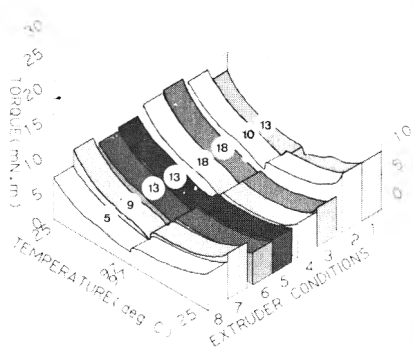
Fig. 2—Viscosity profiles and degree of cook values of extruded starch (OSVA series). Encricled numbers represent amount of uncooked starch.

extruded under steady state conditions as monitored by the torque output from the drive motor. As temperature of metering zone or the die was changed during any run, the extruder was allowed to operate until the torque output indicated a new but constant level. At this point, samples were taken and retained for further examination.

Extrudates were collected from the 6.25 mm circular rod die, cut into 2.5–5 cm lenghs and immediately immersed in liquid N_2. The frozen extrudates were ground in a coffee mill to pass a 0.177 mm screen and air dried before using for functional paste viscosity measurement according to Paton and Spratt (1978).

The functional viscosity profiles of powdered extrudates were determined on an Ottawa Starch Viscometer (OSV) according to Voisey et al. (1977). This is a novel, rapid recording viscometer which produces pasting curves in 10% of the time and uses 10% of the material normally required with a Brabender Amylograph. All samples were analyzed on a 9% (dm) solids starch basis. Each powdered extrudate was carefully blended into the required amount of distilled water using a modified Virtis tissue homogenizer to ensure a lump-free smooth dispersion (Paton and Spratt, 1978). Each slurry was then adjusted to pH 6.8, poured into the viscometer bowl and then allowed to stand for 30 min before inserting the bowl into the viscometer. This was to allow time for recovery of any viscosity which may have been partially lost during the period of dispersion in the Virtis homogenizer. Each slurry was then pasted in the OSV by heat transfer as the sample bowl rapidly rotated in the water bath (97°C);. Once the sample had reached 97°C, and after a brief (3 min) holding period at this temperature, the hot water in the bath was quickly drained and replaced by running tap water at 10–12°C in order to cool the hot paste to 25°C. The resulting curve (Fig. 1) is a direct recording of torque (viscosity or consistency) against the temperature of the slurry.

For each extruded product, the area ABC (Fig. 1) bounded by the Y axis and the intersection of the heating and cooling curves, is measured and the amount of uncooked starch is read from a calibration curve. This curve is constructed by running paste viscosity profiles on known mixtures of unprocessed and precooked wheat starch and plotting the enclosed loop areas as a function of mixture composition (Paton and Spratt, 1981).

In order to assess the influence of a particular extrusion condition upon wheat flour and its composites, the consistency or viscosity curves were treated by a computer graphic technique and displayed in 3 dimensional arrays as described by Bishop et al. (1981). In order to show the features of these families of curves to best advantage, it was necessary to digitize each curve and to assemble these in the format illustrated in Fig. 2 through 5. Here the magnitude of the initial consistency (viscosity) is displayed in the foreground at 25°C. The heating portion of the curve is then displayed as a function of temperature in the range 25–97°C. following a short holding time (3 min) at 97°C, the contents of the viscometer bowl are cooled from 97 to 25°C; the final consistency of the cooked and cooled paste is thus displayed towards the background of each figure.

The Expansion Index (EI) of each extrudate was calculated as the ratio of the cross sectional areas of the product and the die respectively. Water Absorption Index (WAI) and Water Solubility Index (WSI) of wheat starch extrudates were determined according to Anderson et al. (1969). Powdered extrudate (3g) were mixed with the aid of a glass rod in a thick-walled tapered centrifuge tube with 30 mL of distilled water. Care was taken to avoid lumping in order to produce a smooth dispersion. Tubes were centrifuged at 2000g for 15 min and the supernatants carefully decanted. The amount of solids in this soluble phase was determined on a 2 mL aliquot by vacuum drying at 110°C for 1 hr. The residue in each decanted tube was weighed and the Water Absorption Index (WAI) was calculated as

STARCH – GLUTEN
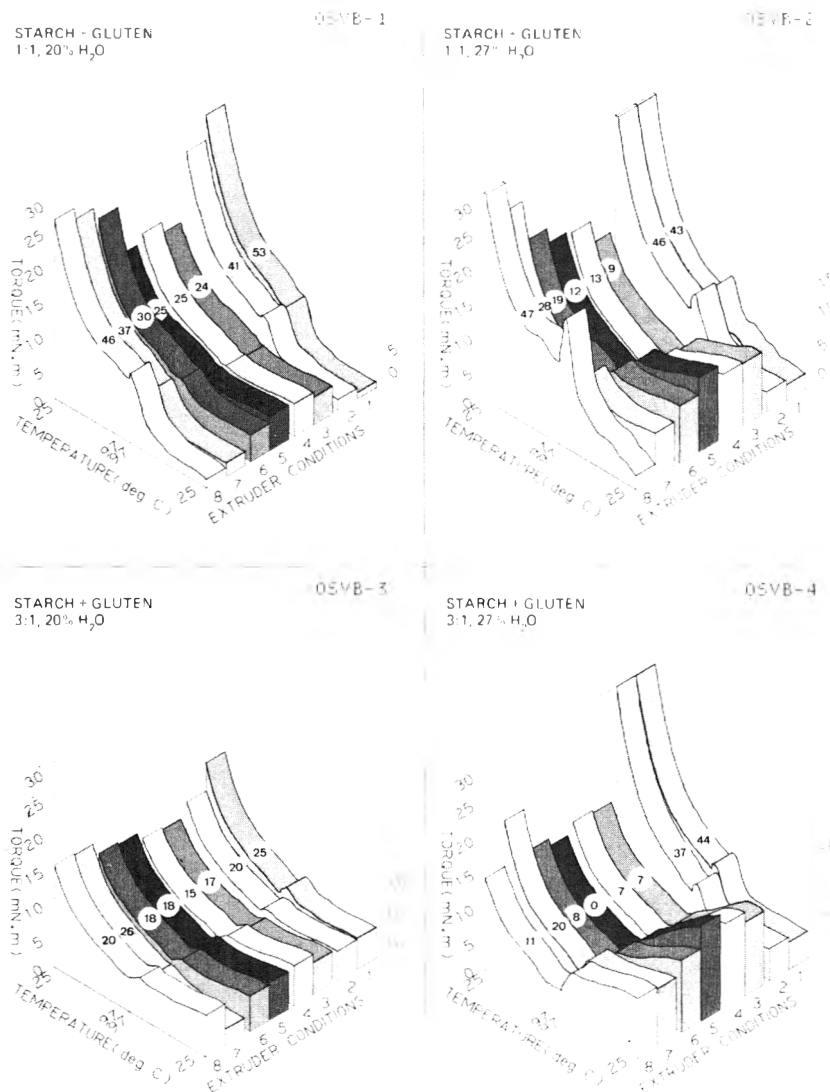1:1, 20% H₂O

STARCH – GLUTEN
1:1, 27% H₂O

Fig. 3—Viscosity profiles and degree of cook values of extruded starch + gluten (OSVB series). Encircled numbers represent amount of uncooked starch.

STARCH + GLUTEN
3:1, 20% H₂O

STARCH + GLUTEN
3:1, 27% H₂O

the ratio of wet residue to starting material, corrected for the amount lost to the soluble phase.

RESULTS & DISCUSSION

Functional viscosity

Fig. 2 through 5 represent the viscosity or pasting profiles of the various extrudates. The foreground vertical axis represents the initial cold paste viscosity and the encircled numbers on each plot represent the amount of uncooked starch. The maximum initial cold paste viscosity for starch was achieved at 100 rpm with a 3:1 screw at 27% H_2O and a metering zone and die temperature of 163° and 120°C, respectively (Fig. 2 Plot OSVA-4 condition 4). Increasing the temperature and screw speed did not always lead to an improvement in the extent of cook of the starch component. At 50 rpm raising the die temperature from 120 to 163°C resulted in a slight increase (16% vs 11%) in the amount of uncooked starch (condition 5 vs 3). At 100 rpm, the amount of uncooked starch was increased from 0 to 15% (condition 4 vs 6). It should also be noted that not all curves analyzing the same amount of uncooked starch have identical paste viscosity profiles, e.g. OSVA-1-5 and OSVA-4-5. Also, lower observed values for the amount of residual uncooked starch should not be equated with improvements in functional paste viscosity (OSVA-3-8 and OSVA-4-3). Recent research by Davidson et al. (1983) has shown such apparent anomalies to be a function of the residence time of the starch within the barrel and to be associated with the variable

shear stresses calculated for the starch undergoing thermal processing.

Fig. 3 illustrates the family of curves for the starch-gluten composites where the gluten has been added at the 10% level and represents the amount of gluten nominally found in the parent soft wheat flour. The starch component of this composite was again found to be most cooked for the 3:1 screw at 27% moisture content (OSVB-4). The initial paste viscosity was higher over a wider range of temperature-screwspeed combinations than was found for wheat starch itself (OSVA-4). However the maximum initial cold paste viscosity (OSVB-4-5) was approximately 25–30% less than that found for wheat starch alone (OSVA-4). Lower temperatures for the OSVB-4 series and most conditions for the OSVB-1 and OSVB-2 series resulted in much less cooking of the starch component as indicated by higher numerical encircled values. This likely reflects the strong association of gluten and water during the pre-mixing stage and the extent to which the water became available to the starch during the extrusion process. A comparison of OSVA-2 would seem to point to a degree of protection by gluten of the starch to the extent that the latter is substantially less cooked.

Fig. 4 illustrates the series of curves obtained for the extrusion of the full composite containing starch, gluten and wheat flour solubles. Apart from the 1:1, 20% moisture series (OSVC-1), cooking of the starch component was very variable over a broad range of conditions. Complete cooking of starch was achieved

STARCH + GLUTEN + SOLUBLES
1:1, 20% H₂O
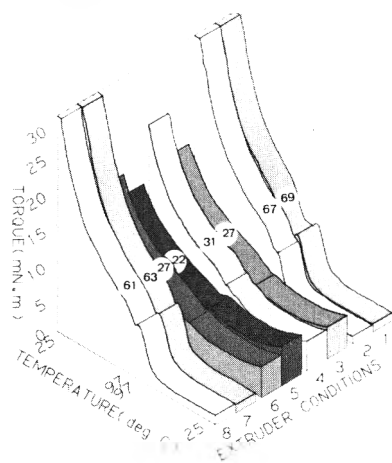
STARCH + GLUTEN + SOLUBLES
1:1, 27% H₂O
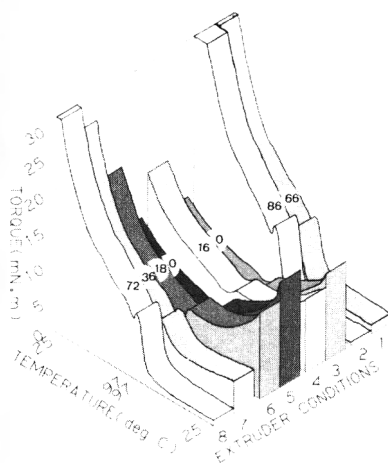
STARCH + GLUTEN + SOLUBLES
3:1, 20% H₂O
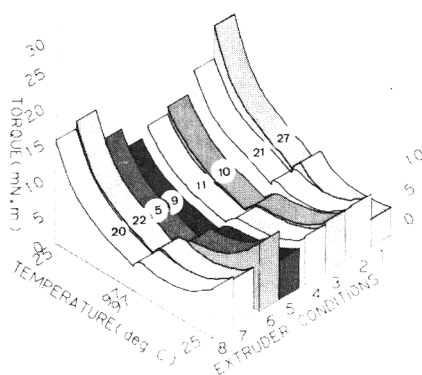
STARCH + GLUTEN + SOLUBLES
3:1, 27% H₂O
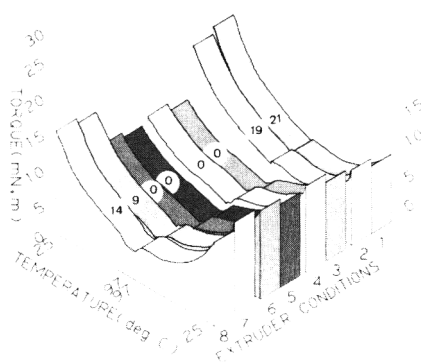
Fig. 4—Viscosity profiles and degree of cook values of extruded starch + gluten + wheat flour solubles (OSVC series). Encircled numbers represent amount of uncooked starch.

even with a 1:1 screw at 27% moisture providing that the temperature of the metering zone was at 163°C. However, increasing the screw speed from 50 to 100 rpm at this temperature, resulted in 16–18% uncooked starch in the product (OSVC-2-4 and OSVC-2-6). This probably reflects a shorter residence time in the barrel. The most striking differences occurred for the OSVC-4 series where greater than 80% of the starch component was cooked regardless of the range of extrusion conditions employed.

These results are in marked contrast to those found for soft wheat flour itself (Fig. 5). Moderate cooking of the starch component is only found for the OSVD-4 group although the extent of cooking was quite variable. Comparison of Fig. 3 (OSVC-4) and Fig. 5 (OSVD-4) clearly illustrates the resistance of wheat flour to cooking over the range of extrusion conditions employed in the current experiments. Such differences very likely reflect the integral structure of the wheat flour particle in terms of the distribution of water during the process. It seems reasonable to assume that the less ordered association of the components in the full composite (Fig. 4, OSVC-4) resulted in easier penetration of water into the starch granules, thereby facilitating the cooking process.

Expansion, water absorption and solubility

The expansion indices (EI) of selected extrudates are shown in Fig. 6. For ease of presentation, only those extrudates prepared under isothermal barrel temperature conditions are given, i.e. where

both the metering zone and the die were at the same temperature, viz. 120°C or 163°C, respectively. For starch alone, the EI increased as the moisture content decreased and the screw speed increased. This effect was more pronounced with the 3:1 compression screw (unshaded columns). The addition of gluten to starch and maintaining the same extrusion conditions, produced quite different results. For a starch-gluten blend at 20% moisture content, 50 rpm and 163°C, both a 1:1 and 3:1 screw produced higher EI values in comparison to wheat starch alone. As the moisture content was increased, the EI decreased which probably reflects the increased hydration and development of elasticity by the gluten component. This probably results in less water available for the starch granules to swell and cook. The addition of wheat flour solubles to a mixture of starch and gluten further lowered the EI of the extrudate. The foregoing data is in marked contrast to the behavior of wheat flour itself. Here the EI is much lower over the entire range of extrusion cooking conditions, the maximum value being 52%, 45% and 63%, respectively, of the maximum EI values for wheat starch, starch + gluten and starch + gluten + solubles.

Fig. 7 (A,B) illustrates the water absorption index (WAI) and water solubility index (WSI) for wheat starch extrudates. It is of little value to examine the WAI and WSI values for either the starch-gluten or full composite systems due to the expected varying solubility of the added protein components. These factors would undoubtedly confound the measurement of these indices. For wheat

Fig. 5—Viscosity profiles and degree of cook values of extruded soft wheat flour (OSVD series). Encircled numbers represent amount of uncooked starch.

Fig. 6—Expansion Indices (EI) for starch + gluten, starch + gluten + wheat flour solubles and wheat flour at different extruder combinations of moisture, isothermal barrel temperature, screw speed and screw compression.

1 — 50 rpm, isothermal temp = 120°C
2 — 100 rpm, isothermal temp = 120°C
5 — 50 rpm, isothermal temp = 163°C
6 — 100 rpm, isothermal temp = 163°C

■ 1:1 20% H$_2$O
□ 3:1 20% H$_2$O
□ 1:1 27% H$_2$O
▨ 3:1 27% H$_2$O

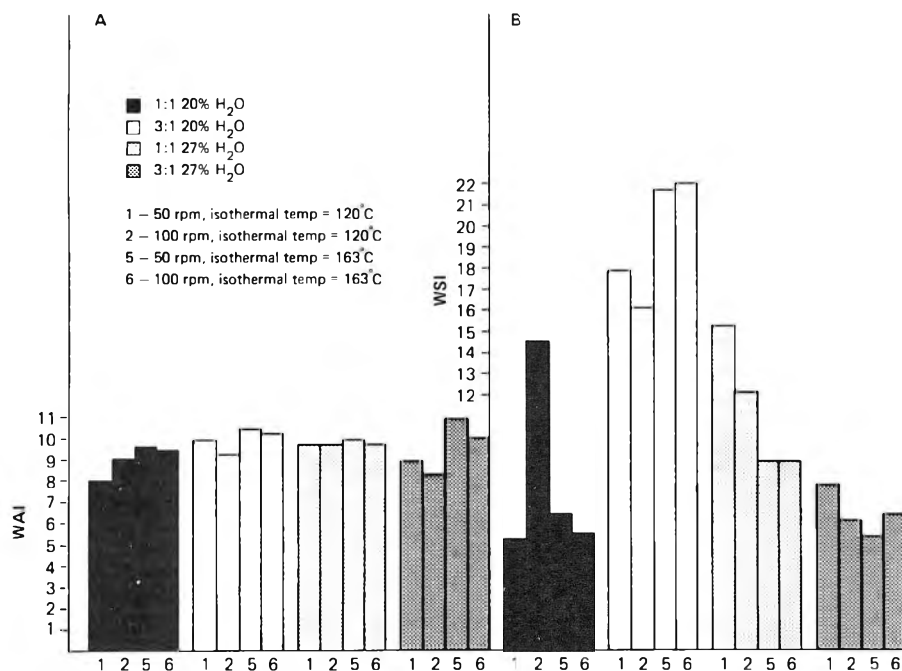*Fig. 7—(A) Water Absorption Indices (WAI) and (B) Water Solubility Indices (WSI) for wheat starch at different extruder combinations of moisture, isothermal barrel temperature, screw speed and screw compression.*

starch, no apparent difference in WAI was observed over the entire range of extrusion conditions examined (Fig. 7A); on the other hand, the WSI pattern was closely associated with variation in feed moisture content of the starch. As moisture content decreased, WSI increased, which is in agreement with results reported by others for pure starches (Mercier and Feillet, 1975) and for corn grits (Conway, 1971). Comparison of Fig. 6 and 7 (starch) with Fig. 2 (OSVA-3 and OSVA-4) would indicate that although expansion index and solubility index are closely related, these in turn are inversely related to functional viscosity and unrelated to degree of cook. Thus such parameters should be used with caution to distinguish among samples in relation to the effectiveness of the cooking process.

The chemistry and rheology of extruded starch systems is not well understood or described in the literature. Colorna and Mercier (1982) have shown with manioc starch that the water soluble fraction of starch extrudates was derived from the amylopectin component of the starch. These authors imply that this fraction imparts a measure of stability to the aqueous dispersions of such extrudates. Davidson et al. (1984) in a separate study of extruded wheat starch, have shown a progressive reduction in the amount of amylopectin component, measured by gel permeation chromatography, as a function of barrel temperature, screw compression ratio and moisture content of the feed starch. One might expect that the structural characteristics of extruded starch species would influence the rheological nature of the functional viscosity of starch extrudates. Although the actual gel strengths of extruded starch pastes were not measured in the present study, we did observe that some pastes from the OSVB and OSVC groups were stable at room temperature for several days and remained salve-like for up to 2 days at 2°C. None of the extruded wheat flour pastes displayed this characteristic.

Attempts to "force" the wheat flour toward a greater degree of cook or improved functional viscosity performance by further raising the temperature, screw speed or moisture content of the feed, only resulted in less apparent cook, greater degradation of the starch component or both. No doubt improvements in paste functionality may be brought about by more sophisticated changes in extruder screw design or die configuration. Davidson et al. (1984), in unpublished work, have shown that structural modifications to starch are a function of the product of the shear stress, calculated to occur in the metering zone of the extruder, and the residence time within the barrel.

No attempt was made in the present study to evaluate the nature and extent of modification of either the gluten or soluble components added to the wheat starch, since to do so would have introduced a further number of variables to an already complex

system. No doubt such factors must be taken into account when determining the functional behavior of such composites. The data presented here are not intended to define what might happen in all types of extruders; it is recognized that specific extruder design will likely play a major role in determining the nature, shape and functionality of extrudates. Rather, the purpose of the present study was to examine what might likely happen in an extruder as one varies the formulation and the extruder process variables and to attempt to relate these in turn to the degree of cooking and functional behavior of the starch component of the resulting extrudates.

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Response Surface Methodology Approach to the Optimization of Boneless Ham Yield

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ABSTRACT

Three ham processing variables (tumbling, tenderization, and temperature) were optimized for cooked yield of both pre- and post-rigor ham muscle (semimembranous). Through response surface methodology (RSM), it was determined that the optimum tenderization (80–100% of knife blade capacity) and processing temperature (14–16°C) conditions were approximately the same for both pre- and post-rigor muscle. However, the optimum level of continuous vacuum tumbling (total revolutions) was much lower for pre-rigor muscle (750 rev) than post-rigor muscle (3500–4000 rev).

INTRODUCTION

CURRENT RESEARCH in the area of boneless ham processing has evaluated the influence of tumbling and associated processing variables (salt, phosphates, fat, cooking temperature, time, postmortem rigor, and tenderization). These studies have attempted to gain an understanding of the physical, chemical, and histological changes associated with tumbled meats (Cassidy et al., 1978; Krause et al., 1978; Motycka and Bechtel, 1983; Ockerman et al., 1978; Siegel et al., 1978; Theno et al., 1977). From these studies, it is evident that cooked ham characteristics are dependent upon a number of variables and their possible interactions. Previous ham processing studies have used conventional statistical analysis techniques (regression, analysis of variance and factorial designs). However, when many variables and their possible interactions are affecting a system, an experimental optimization approach knows as Response Surface Methodology (RSM) is appropriate (Davies, 1956; Grethlein, 1977).

Through planned experimentation, RSM is aimed at specifically identifying variables effects and seeking conditions which optimize the response in question (Box et al., 1978). This statistical technique has been used sparingly by food scientists (Lah et al., 1980), but has been extensively used in other areas of engineering and chemical science. Application of RSM to boneless ham processing systems offers promise in determining optimum processing conditions.

Most previous research on ham processing has utilized post-rigor meat except for Solomon (1979) and Motycka and Bechtel (1983). Differences in pre- and post-rigor processing characteristics are evident (Cassens, 1966; Newbold, 1966; Pearson, 1971), but the magnitude of these differences when subjected to varying processing techniques and levels has not been evaluated. Therefore, this study was designed to: (1) apply RSM to a boneless ham processing system in which three processing variables (tumbling, tenderization and temperature) are optimized for cooked yield, and (2) compare pre- and post-rigor ham processing characteristics.

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MATERIALS & METHODS

Response Surface Methodology (RSM)

eight market weight hogs were conventionally slaughtered with the resulting 16 semimembranous muscles randomly assigned by animal to one of eight (2^3 factorial design) pre- and post-rigor processing treatments (tumbling revolutions = 3000, 4000; percent mechanical tenderization = 100, 150; processing temperature, °C = 8, 16). To gain information at the center point of the 2^3 factorial design and to insure that our experimental animal population was homogeneous for the cooked yield response, eight additional market weight hogs were slaughtered. Semimembranosus muscles from four animals (two replicates per animal) were randomly allotted to each pre- and post-rigor processing treatment (tumbling revolutions = 3500; percent mechanical tenderization = 125; processing temperature = 12°C), i.e., eight replicates for each pre- and post-rigor treatment. This information was used to assess the statistical significance of data obtained from the initial 2^3 factorial design.

From the results of the initial 2^3 factorial and replicated center point experimentation, pre-rigor RSM required experimentation along the "path of steepest ascent" (PSA) for increased cooked yield. One market weight hog was slaughtered with resulting semimembranosus muscles processed according to a PSA determination (tumbling revolutions = 3432; percent mechanical tenderization = 75; processing temperature = 21°C).

The cooked yield result from the PSA test indicated movement away from previously determined large cooked yield values. A second order design experiment (3^2 factorial) was now appropriate to further define the response surface in this area. The 3^2 factorial design required nine experimental tests; however, two tests were previously performed in connection with the 2^3 factorial and PSA experiments. Therefore, seven market weight hogs were slaughtered with the resulting 14 semimembranosus muscles randomly allotted by animal to one of seven remaining 3^2 factorial treatments (tumbling revolutions = 3500; percent mechanical tenderization = 75, 100, 125; processing temperature °C = 12, 16, 21). To finally clarify the optimum pre-rigor cooked yield response surface in the direction of reduced tumbling revolutions, sixteen market weight hogs were slaughtered. Semimembranous muscles from four animals (one animal per replicate) were randomly allotted to each of four tumbling revolutions treatments (0, 750, 1500, 3000). Percent mechanical tenderization (100) and processing temperature (16°C) were held constant at their established optimum levels.

From the results of the initial experimentation, post-rigor RSM required a second order central composite design (5 levels for three variables) experiment to further define the response surface in this area. Since the central composite design augmented the initial experimentation, six additional tests were required. Therefore, six market weight hogs were slaughtered with the resulting twelve semimembranous muscles randomly allotted by animal to one of six remaining central composite treatments (tumbling revolutions = 2659, 3500, 4341; percent mechanical tenderization = 83, 125, 167; processing temperature °C = 4.3, 12, 18.7).

Processing

All market weight hogs were conventionally slaughtered and similar in genetic and management background. Pre-rigor ham muscles were removed from the carcass immediately after slaughter (less than 10 min. after stunning) and processed. Post-rigor hams remained intact and were chilled at 2 ± 2°C for 24 hours. The ham muscles were then removed and stored at 20°C until their internal temperature equalled their ultimate processing temperature. Subcutaneous and intermuscular fat and connective tissue was removed. Due to difficulty in accurately separating the adductor muscle from

the semimembranous muscle, the two muscles remained joined and were considered as the semimembranous muscle. Individual muscles were multineedle injected by hand to 20% of original meat weight with a curing solution [12.4% salt, (minimum 2% in finished product) 2.5% phosphate mixture (Curafos), 0.08% sodium nitrite, 3.7% dextrose and 81.32% water], tenderized, cut in half (parallel with muscle fibers), and continuously tumbled. After tumbling, individual muscles (two halves of each muscle) were weighed and placed in cellulose casings, vacuum packaged in a cooking pouch, cooked to an internal temperature of 72 ± 2°C, and chilled to an internal temperature of 2 ± 2°C. Each muscle was removed from the casing, allowed to drain for 10 minutes, and weighed. Cooking yield was calculated: % cooked yield = (wt of muscle after cooking/wt of muscle before cooking) × 100. Visual and palatability evaluations of samples which exhibited the highest cooked yields were performed informally to assure acceptable quality.

Mechanical tenderization

A Model IT2 Belam Mechanical Tenderizer (Belam, Inc., Downers Grove, IL) was used to tenderize the meat. This machine has two horizontal, parallel shafts each containing 65 circular, variegated knife blades spaced 5 mm apart. The knife blades rotate towards one another, thus drawing meat chunks down past the knife blades. The distance between the outside edges of the knife blades from each shaft is less than 1 cm; therefore, meat chunks receive cuts 5 mm apart and within 1 cm of being completely separated into smaller pieces. Muscles were individually tenderized with initial cuts occurring perpendicular to the long axis of the muscle fibers. When the amount of tenderization required was more than provided by the knife blades normally present (100%), the meat chunks (muscle fibers parallel with knife blades) were passed through the machine a second time with a reduced number of knife blades present.

Tumbling and cooking

The tumbling of the meat chunks was accomplished with a Universal 190 Inject Star Tumbler (Globus Labs, Hackensack, NJ) operated continuously with vacuum (584–660 mm Hg gauge). After tumbling, individual muscles (two halves of the same muscle) were weighed, placed into a 12.5 cm diameter easy peel, prestuck, fibrous casing (Teepak, Danville, IL), vacuum packaged (Kenfield Model C-14, International Kenfield, Park Ridge, IL) in a cooking pouch, placed in a hot water bath (75 ± 2°C) for approximately 150 minutes to an internal temperature of 72 ± 2°C, and then chilled for 24 hr to an internal temperature of 2 ± 2°C.

RESULTS & DISCUSSION

RSM DETERMINES optimum conditions by combining special experimental designs with modeling by first and second order polynomial equations in a sequential testing procedure. Initially in this study, a 2^3 factorial experiment with replicated center points was performed for both pre- and post-rigor muscle (Table 1). With this information, estimates of the linear coefficients for a first order equation $[Y = b_0 + b_1 X_1 + b_2 X_2 + b_3 X_3$ (an approximation of the response surface in the area of experimentation)] were determined (Table 2). Fitted prediction equations for the cooked yield response of pre-rigor (Y_1) and post-rigor (Y_2) semimembranous muscle were determined by substituting the coded independent variable values (X) and their estimated coefficients (b) into the first order equation:

$$Y_1 = 89.85 - 0.6\, X_1 - 0.88\, X_2 + 0.99\, X_3$$
$$Y_2 = 86.87 + 1.04\, X_1 - 2.15\, X_2 - 0.35\, X_3$$

$(X_1$ = tumbling revolutions; X_2 = percent mechanical tenderization; X_3 = processing temperature). These fitted prediction equations (linear models) determine the direction and magnitude of variable adjustment to move into the region of optimum yield, i.e., determine the path of steepest ascent (PSA). They are based upon the assumption that

Table 1—Cooked yield results from a 2^3 factorial experiment plus four center points utilizing pre- and post-rigor semimembranous muscles

| Treatments (Equation symbol) | | | % Cooked yield[d] (Y) | | | |
| | | | Pre-rigor | | Post-rigor | |
Tumble[a] (X_1)	Mech[b] tender (X_2)	Temp[c] (X_3)	Exp. order	Semi-membranous	Exp. order	Semi-membranous
3000 (−1)	100 (−1)	8 (−1)	4	90.72	2	84.24
4000 (+1)	100 (−1)	8 (−1)	8	88.05	1	92.08
3000 (−1)	150 (+1)	8 (−1)	7	87.14	8	86.85
4000 (+1)	150 (+1)	8 (−1)	3	89.52	5	85.25
3000 (−1)	100 (−1)	16 (+1)	1	92.60	3	90.64
4000 (+1)	100 (−1)	16 (+1)	6	91.52	6	88.68
3000 (−1)	150 (+1)	16 (+1)	2	89.15	7	81.15
4000 (+1)	150 (+1)	16 (+1)	5	90.07	4	85.15
3500 (0)	125 (0)	12 (0)	9	90.25	9	84.50
3500 (0)	125 (0)	12 (0)	10	91.30	10	88.32
3500 (0)	125 (0)	12 (0)	11	89.68	11	87.60
3500 (0)	125 (0)	12 (0)	12	90.46	12	90.30

[a] Total continuous tumbling revolutions: 28 rpm at 635 millimeters Hg vacuum in Inject Star Tumbler (Model 190).
[b] Percent mechanical tenderization: Belam Mechanical (Knife) Tenderizer (Model IT2).
[c] Processing room and internal meat temperature (°C).
[d] Percent cooked yield = $\dfrac{\text{wt of muscle after cooking}}{\text{wt of muscle before cooking}}$ × 100.

Table 2—Linear coefficients for tumbling revolutions, percent mechanical tenderization, processing temperature, and interactions[a]

| % Cooked yield | Mean b_0 | Tumble b_1 | Mech tender b_2 | Temp b_3 | Interactions | | | |
					b_{12}	b_{13}	b_{23}	b_{123}
Pre-rigor semimembranous[b] Y_1	89.85	−0.06	−0.88	0.99	0.88	0.02	−0.34	−0.38
Post-rigor semimembranous[c] Y_2	86.87	1.04	−2.15	−0.35	−0.43	−0.52	1.10	1.92

[a] Calculated according to Box et al., 1978.
[b] 95% confidence Interval for b_i = ±0.97
[c] 95% confidence interval for b_i = ±1.94

the effects of the variables are additive. However, significant interaction between variables would indicate non-additivity and the inadequacy of using a linear model to approximate the response surface.

In checking the interaction of the variables as measured by the estimated coefficients, $b_{12} = 0.88$, $b_{13} = 0.02$, $b_{23} = -0.34$ and $b_{123} = -0.38$ for the pre-rigor cooked yield response (Y_1), there is evidence of additivity since a 95% confidence interval for the true value of the interaction effect parameters includes zero for each case above. For the post-rigor cooked yield response (Y_2), the check of estimated interaction coefficients ($b_{12} = -0.43$, $b_{13} = -0.52$, $b_{23} = 1.10$, and $b_{123} = 1.92$) reveals some indication of curvature (nonadditivity) since the interaction coefficients ($b_{23} = 1.10$ and $b_{123} = 1.92$) are generally larger in magnitude than the first order coefficients and of the magnitude of two standard errors for effects. Thus, the pre-rigor linear model was an adequate approximation of the response surface; whereas, the post-rigor linear model was not. Therefore, continued pre-rigor RSM experimentation was in the direction of the predicted optimum cooked yield area; whereas, post-rigor RSM required second order design experimentation to further examine the nature of curvature in the initial response surface area.

For pre-rigor RSM, the equation Y_1 was used to define the "Path of Steepest Ascent" (PSA). Experimentation along the PSA for pre-rigor semimembranous muscle (Table 3, experiment number 13) resulted in a lower cooked yield (90.3). With larger cooked yields of 92.6 and 91.5 previously observed on either side of the PSA, this indicated a movement away from a large cooked yield response region. Therefore, to further examine the response surface in this area, a second order design (3^2 factorial) was used to augment the last first order design. A 3^2 factorial design was selected because an experimental design with more than two levels of each factor is required so that a second order approximation of the response surface can be determined: $Y = b_0 + b_2 X_2 + b_3 X_3 + b_{23} X_2 X_3 + b_{22} X_2^2 + b_{33} X_3^2$. Due to the small effect of tumbling revolutions, this variable was fixed at its previous center point value of 3500. The cooked yields from the 3^2 factorial experiment for the pre-rigor semimembranous muscle are shown in Table 3. The coefficients for the cooked yield response (second order model) Y_1 are given: $Y_1 = 41.14 + 0.44X_2 + 3.76X_3 - 0.01X_2 Y_3 - 0.001X_2^2 - 0.09X_3^2$. The analysis of variance for Y_1 indicated that the model was significant ($P < 0.01$). The model accounted for 62.6% ($R^2_{corrected} = 0.62619$) of the

total variation after being corrected for the mean. A contour plot representation of the percent cooked yield response surface for the pre-rigor semimembranous muscle is shown in Fig. 1. This plot indicates that the percent cooked yield response is less sensitive to changes in mechanical tenderization than to changes in processing temperature in these ranges. With tumbling revolutions having little influence on the cooked yield response in this region of the response surface, additional experimentation in the direction of decreased tumbling revolutions (Table 4) indicated that the optimum pre-rigor cooked yield was achieved with minimum tumbling revolutions = 750, percent mechanical tenderization = 100, and processing temperature = $16°C$. The cooked yield response surface for tumbling revolutions appears to be flat in the area of 750-3000 revolutions.

For post-rigor optimization of cooked yield, RSM required second order design experimentation to further evaluate the curvature in the initial response surface area. A central composite design (5 levels for three variables) experiment with the center located at the center points of the initial 2^3 design was performed (Table 5). The coefficients for the cooked yield response (second order model) Y_2 is given: $Y_2 = 13.70 + 0.03X_1 + 0.13X_2 + 3.22X_3 - 0.0003X_1 X_2 - 0.003X_1 X_3 - 0.01X_2 X_3 - 0.00003X_1^2 + 0.003X_2^2 - 0.05X_3^2$ (X_1 = tumbling revolutions; X_2 = percent mechanical tenderization; X_3 = processing temperature). The analysis of variance for Y_2 indicated that the model was significant ($P < 0.01$). The model accounted for 44.89% ($R^2_{corrected} = 0.44892$) of the total variation after
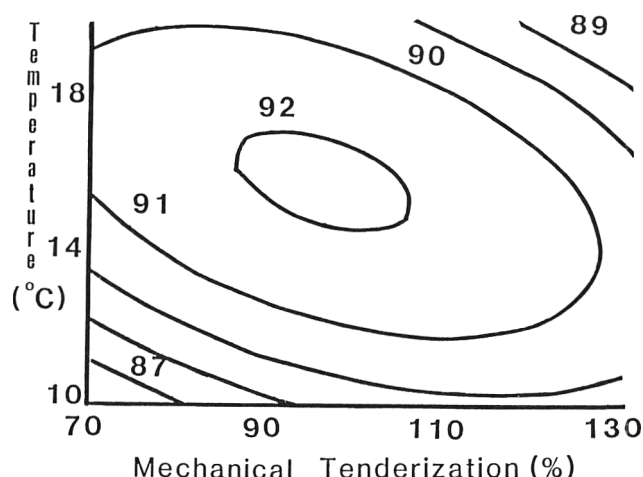
Fig. 1—A contour plot representation of the percent cooked yield for pre-rigor semimembranous muscle with tumbling revolutions = 3500.

Table 3—Cooked yield results from a 3^2 factorial experiment for pre-rigor semimembranous muscle

Treatments (Equation symbol)				
Tumble[a] (X_1)	Mech[b] tender (X_2)	Temp[c] (X_3)	Exp order	Cooked[d] yield (Y)
3500	125	12	9,10,11,12	90.43 (avg)
3432	75	21	13	90.31
3500	100	16	20	92.60
3500	75	12	14	91.15
3500	75	16	15	91.19
3500	100	12	16	89.32
3500	125	16	17	90.66
3500	125	21	18	87.63
3500	100	21	19	91.53

[a] Total continuous tumbling revolutions: 28 rpm at 635 millimeters Hg vacuum in Inject Star Tumbler (Model 190).
[b] Percent mechanical tenderization: Belam Mechanical (Knife) Tenderizer (Model IT2).
[c] Processing room temperature (°C).
[d] Percent cooked yield = $\frac{\text{wt of muscle after cooking}}{\text{wt of muscle before cooking}} \times 100$.

Table 4—Means and standard errors for the effect of tumbling revolutions on cooked yield and binding strength of cured, tenderized,[a] tumbled, and cooked pre-rigor semimembranous muscle

Tumbling[b] revolutions	Obser-vations	Cooked[c] yield (%)	S.E.	Binding[d] strength (g/cm²)	S.E.
0	4	78.34[e]	3.62	103.6[e]	20.06
750	4	88.51[f]	1.31	202.3[f]	45.91
1500	4	87.93[f]	1.74	259.1[f]	45.18
3000	4	90.60[f]	1.09	230.1[f]	28.12

[a] Mechanical tenderization: Belam Mechanical (Knife) Tenderizer Model IT2).
[b] Total continuous tumbling revolutions: 28 rpm at 635 millimeters Hg vacuum in Inject Star Tumbler (Model 190).
[c] Percent cooked yield = $\frac{\text{wt of muscle after cooking}}{\text{wt of muscle before cooking}} \times 100$.
[d] Measured on an Instrom Universal Testing Machine in g/cm².
[e,f] Means bearing different letters in the same column are different ($P < 0.05$).

Fig. 2—A contour plot representation of the percent cooked yield response for post-rigor semimembranous muscle with percent mechanical tenderization = 80.
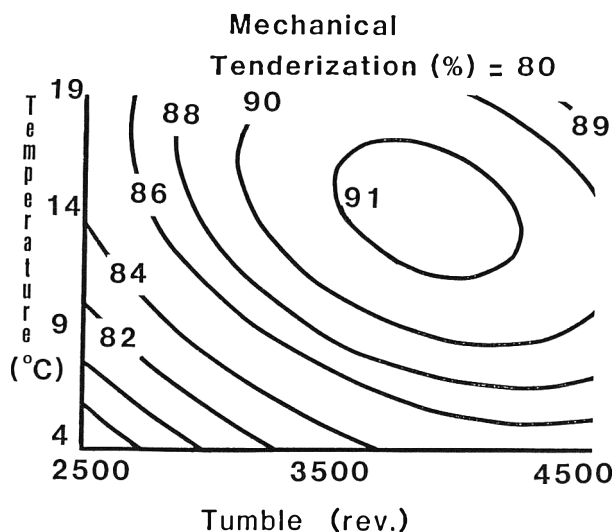

Fig. 3—A contour plot representation of the percent yield response for post-rigor semimembranous muscle with temperature = 16° C.

Table 5—Cooked yield results from the central composite star point tests for post-rigor semimembranous muscle

Treatments (Equation symbol)				
Tumble[a] (X_1)	Mech[b] tender (X_2)	Temp[c] (X_3)	Exp order	Cooked[d] yield (Y_2)
2659	125	12	2	85.51
4351	125	12	3	84.58
3500	83	12	5	87.64
3500	167	12	6	87.51
3500	125	4.3	1	86.56
3500	125	18.7	4	82.75

[a] Total continuous tumbling revolutions: 28 rpm at 635 millimeters Hg vacuum in Inject Star Tumbler (Model 190).
[b] Percent mechanical tenderization: Belam Mechanical (Knife) Tenderizer (Model IT2).
[c] Processing room and muscle temperature (°C).
[d] Percent cooked yield = $\dfrac{\text{wt of muscle after cooking}}{\text{wt of muscle before cooking}} \times 100$.

being corrected for the mean. A contour plot (Fig. 2) represents the response surface in the tumble, temperature dimensions for a constant level of mechanical tenderization = 80%. Similar plots for varying values of mechanical tenderization (e.g. 70, 90) showed that the optimum lies near the 80% value. This can be seen by examining Fig. 3.

In conclusion, it was found that the optimum mechanical tenderization level (80–100% of knife blade capacity) and processing temperature (14–16°C) conditions were approximately the same for both pre- and post-rigor muscle conditions. However, the optimum level of continuous
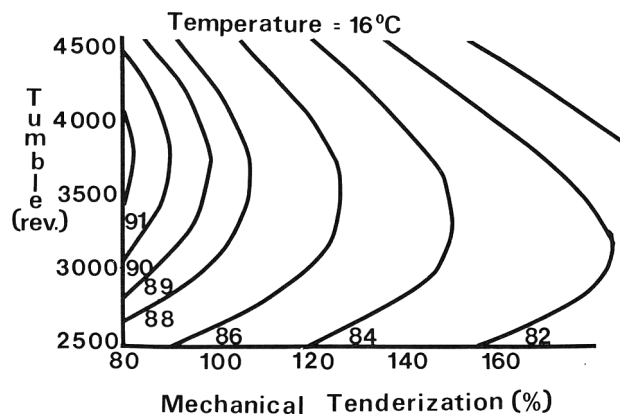
vacuum tumbling (total revolutions) was much lower for pre-rigor muscle (750 rev) than post-rigor muscle (3500–4000 rev).

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A Research Note
Distribution of Cholesterol in Fractionated Beef Tallow

T. C. RYAN and J. I. GRAY

———————————— ABSTRACT ————————————

The cholesterol contents of tallow and tallow fractions derived by aqueous detergent fractionation were determined by gas chromatography. Tallow was found to contain 0.14% cholesterol by weight. Liquid and solid fractions subsequently derived by temperature fractionation at 40, 33, 27, 22 and 18°C contained between 0.10 and 0.15% cholesterol by weight. The liquid fraction at each temperature had cholesterol contents 0.02 – 0.04% higher than the corresponding solid fractions.

INTRODUCTION

AQUEOUS DETERGENT FRACTIONATION of edible beef tallow yields fractions which could have commercial applications as hardening agents, shortening, cocoa butter substitutes and cooking oils (Bussey et al., 1981). Several of these fractions have been investigated as frying media, while some were utilized as shortenings in the manufacture of cookies and cakes (DeFouw, 1981). The development of a fraction which could be used as a pourable shortening for deep-fat frying is desirable because of its ability to impart desirable tallow-fried flavors to the finished fried products.

Unfractionated beef tallow contains 0.15 – 0.20% cholesterol by weight (Punwar and Derse,1978). Recent studies have indicated that cholesterol present in tallow subjected to deep-fat frying conditions (180°C) is prone to oxidative deterioration (Ryan, 1982; Ryan et al., 1981). Unfortunately, some oxidative derivatives of cholesterol have been implicated as being angiotoxic (Imai et al., 1976; Peng et al., 1978) and/or carcinogenic (Bischoff, 1969; Smith and Kulig, 1975). Thus, the major purpose of this study was to determine if cholesterol is evenly distributed through the various tallow fractions derived in aqueous detergent fractionation processes, and in particular those that might be used in high temperature food preparations as it would be desirable to achieve a liquid fraction containing less cholesterol than the parent tallow sample.

MATERIALS & METHODS

Beef tallow

A 400 lb drum of refined edible beef tallow was obtained from a commercial supplier and stored at 4°C.

Fractionation of tallow

A modification of the aqueous detergent fractionation method proposed by Bussey et al. (1981) was utilized. Tallow (1 kg) was allowed to soften for 24 hr at 40°C. Sodium dodecyl sulfate (SDS) was subsequently added as a powder to the softened tallow at a level of 0.6% based on tallow weight. The softened tallow was allowed to crystallize for 18 hr at 40°C followed by the addition of 600 mL-5% sodium citrate solution warmed to 40°C. After 1 hr of equilibration at 40°C, the mixture was centrifuged at 1240 x g for 15 min at room temperature. The resulting stearin (solid) frac-

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tion was washed repeatedly with hot H_2O to remove the SDS while the clear olein (liquid) phase was further fractionated at 33, 27, 22 and 18°C as shown in Fig. 1. Additional detergent was not introduced after the initial fractionation at 40°C because the combination of decreasing temperature and centrifugation provided satisfactory separation.

Analysis of fatty acids

Original edible tallow and fractionated tallow samples were methylated by the boron trifluoride-methanol method of Morison and Smith (1964) utilizing the preparation conditions for triglycerides. Analysis of fatty acids (C_{14}-C_{20}) was carried out with a 5840A Hewlett Packard gas chromatograph equipped with a glass column (2 m x 4 mm i.d.) packed with 15% diethylene glycol succinate on 80/100 Chromosorb W (Ackman, 1969). An isothermal program was utilized with column temperature, 190°C; injector temperature, 210°C; flame ionization detector, 300°C; nitrogen (carrier) flow rate at the detector, 40 mL/min.

Analysis of cholesterol

Extraction of cholesterol from tallow. Original edible tallow and fractionated tallow samples (in duplicate) were subjected to saponification and extraction of cholesterol by the method described by Itoh et al. (1973). Ten grams of each tallow sample were refluxed for 1 hr with 100 mL of 1.0N alcoholic KOH. The saponified samples were cooled and 100 ml of distilled H_2O were added to each sample. The nonsaponifiable matter was extracted from the samples using 5 x 100 mL aliquots of isopropyl ether in 1L separatory funnels. The ether extracts for each sample were combined and washed with 5 x 100 mL aliquots of distilled H_2O and then mixed with anhydrous sodium sulfate for 2 hr at 4°C to remove residual water. The ether extracts were filtered, and evaporated to dryness in a Buchi Rotovapor R rotary evaporator (Buchi Inc., Switzerland). The residue was redissolved in ethyl acetate and quantitatively transferred to a 25 mL volumetric flask containing 1.0 mL of a 5 mg/mL stock solution of 5α-cholestane in ethyl acetate used as an internal standard for gas chromatographic (GC) analysis. The flasks were filled to the mark with ethyl actate, flushed with nitrogen, and stored at 4°C until analysis.

Standard cholesterol solutions

A stock solution of cholesterol was prepared by dissolving 250 mg cholesterol in 50 mL of ethyl acetate, yielding a working concentration of 5 mg/mL stock solution.

A series of standard cholesterol solutions was made by pipetting 0, 1, 2, 3, 4, 5, 6, and 7 mL of the stock solution into 25 mL volumetric flasks containing 1.0 mL of the 5.0 mg/mL solution of 5α-cholestane (internal standard), yielding standard solutions of 0, 5, 10, 15, 20, 25, 30, and 35 mg/25 mL.

Quantitation of cholesterol

Cholesterol quantitation was carried out using a 5840A Hewlett Packard gas chromatograph equipped with a glass column (2 m x 4 mm i.d.) packed with 3% SP 2100 on 100-200 mesh Supelcoport (Supelco, Bellafonte Park, PA). An isothermal program was utilized with column temperature, 265°C; injector temperature, 280°C; flame ionization detector temperature, 350°C; nitrogen (carrier) flow rate at the detector, 35 mL/min. The retention times of 5α-cholestane (internal standard) and cholesterol were 4.5 and 7.6 min respectively. Injection volume was 2.5 μL for all standard cholesterol solutions as well as the samples derived from fractionated and original tallow samples.

RESULTS & DISCUSSION

FRACTION YIELD, mg cholesterol, and percent cholesterol by weight for aqueous detergent fractionated tallow are presented in Table 1. Unfractionated tallow was found to contain 0.14% cholesterol by weight while subsequent tallow fractions contained between 0.10 - 0.15% cholesterol. It was also observed that the liquid fraction at each temperature contained slightly more cholesterol than the corresponding solid fraction.
esterol than the corresponding solid fraction.

Cholesterol recoveries ranged from 91 - 102% based on the total cholesterol content (mg) in the liquid phase compared to the sum of cholesterol (mg) in the subsequent solid and liquid fractions. The low cholesterol content (0.10%) observed in the 40°C solid fraction may be attributed to the use of SDS and electrolyte solution in the primary fractionation step (Fig. 1). It is possible the SDS-electrolyte solution may emulsify cholesteryl esters which are subsequently lost in hot water washing techniques needed to remove residual SDS. This is reinforced by the observation that approximately 12g of 40°C solid tallow, representing 5% of this fraction, were also lost in the hot water washing procedure due to emulsification by SDS.

The results of the fatty acid analyses for tallow and tallow fractions are found in Table 1. Original tallow had a saturated/unsaturated fatty acid ratio of 0.85, while subsequent oil fractions had increasingly higher levels of unsaturated fatty acids. Liquid fractions obtained at temperatures of 27°C and lower had saturated/unsaturated fatty acid ratios of approximately 0.65.

The increasing unsaturation of these liquid fractions may have a modifying influence on the extent of cholesterol oxidation when these fractions are heated. Current studies of heated triolein and tristearin systems containing cholesterol have indicated that under static conditions of heating, unsaturated fatty acyl moieties have a sparing effect on the oxidation of cholesterol (Ryan, unpublished data). Unsaturated fatty acids appeared to be preferentially oxidized over cholesterol when the systems were held at 180°C for 2 wk. Thus, the oxidative stability of cholesterol in tallow fractions obtained at the lower fractionation

temperatures may be enhanced by the greater concentration of unsaturated fatty acids associated with these fractions. Studies are currently in progress to determine the effect of degree of fatty acid unsaturation on cholesterol stability in tallow, as well as the protective effect of small amounts (5 - 15%) of cottonseed oil on cholesterol in tallow.
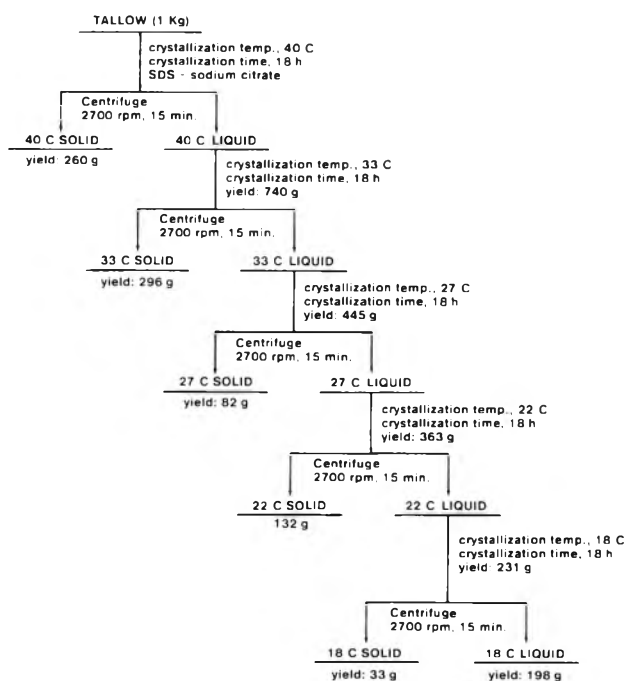
Fig. 1—Fractionation of edible tallow using sodium dodecyl sulfate and sodium citrate.

Table 1—Fraction weight, cholesterol content, and fatty acid composition of edible tallow and tallow fractions obtained by aqueous detergent fractionation

	Original tallow	40°C		33°C		27°C		22°C		18°C	
		Solid	Oil	Solid	Oil	Solid	Oil	Solid	Oil	Solid	Oil
Fraction weight (g)	1,000	247[a]	740[b]	296	445	82[b]	363	132[b]	231	33[b]	198
Total cholesterol (mg)	1,355	259	970	360	611	93	475	152	332	37	290
% Cholesterol	0.14	0.10	0.13	0.12	0.14	0.11	0.13	0.12	0.14	0.11	0.15
Fatty acid composition (%)											
C14:0	3.2	3.0	2.9	3.0	2.8	3.2	2.6	2.7	2.7	2.9	2.6
C16:0	24.6	25.7	23.6	24.5	22.6	24.6	21.4	22.1	21.8	23.2	22.0
C18:0	16.4	20.5	16.3	17.6	15.5	17.0	14.0	14.3	13.4	15.1	13.3
C20:0	0.2	0.1	0.1	0.1	0.1	—	0.2	—	0.1	—	—
C14:1	1.4	1.1	1.3	1.1	1.3	1.3	1.3	1.3	1.3	1.4	1.4
C16:1	4.2	3.8	4.2	4.1	4.3	4.1	4.6	4.5	4.8	4.6	4.9
C18:1	40.5	37.1	42.2	41.2	44.3	40.6	47.6	46.5	47.2	46.0	48.7
C18:2	4.0	3.4	3.8	3.5	3.9	3.7	3.8	3.8	3.8	1.7	2.0
C18:3	2.0	1.8	2.0	1.5	2.0	1.6	1.6	1.6	1.6	1.8	2.0
% Saturated	44.4	49.3	42.9	45.2	41.0	44.8	38.2	39.1	38.0	41.2	37.0
% Unsaturated	52.1	47.2	53.7	51.4	55.8	51.5	58.9	57.7	58.8	55.5	59.0
Ratio S/U	0.85	1.04	0.80	0.88	0.73	0.87	0.65	0.68	0.65	0.74	0.64

[a] Actual amount recovered after hot water extraction to remove SDS.
[b] Solid weight determined by difference.

Oxidation of a Lipid Emulsion by a Peroxidizing Microsomal Fraction from Herring Muscle

BOHDAN M. SLABYJ and HERBERT O. HULTIN

ABSTRACT

A microsomal fraction isolated from herring (*Clupea harengus*) muscle was shown to oxidize the lipids in an emulsion prepared from extracted lipids of herring muscle. The oxidation of the lipids of the emulsion was dependent on enzymic oxidation of the microsomal lipids. In the absence of either the microsomes or NADH (previously shown to be required for enzymic lipid peroxidation), no measurable oxidation occurred in either the lipid fraction of the microsomes or the lipids of the emulsion. Disappearance of eicosapentaenoic and docosahexaenoic acids from peroxidizing assay systems in presence and absence of lipid emulsion corresponded to the increase in TBA reactive substances.

INTRODUCTION

RANCIDITY in fatty fish is a major problem responsible for quality loss during handling and storage. Not only is flavor affected, but lipid oxidation may also cause undesirable changes in texture through an interaction of proteins and the products of lipid oxidation (Reineccius, 1979). Although it has been generally believed that lipid oxidation in fish tissue occurs by strictly chemical processes, Banks (1937) observed that herring tissue had a catalytic effect on lipid oxidation. This catalytic effect was stimulated by sodium chloride and could be destroyed by heat. Similarly, Bosund and Ganrot (1970) observed that lipid oxidation in both herring and cod fillets could be reduced by pre-cooking. The latter workers suggested that the decrease in lipid oxidation could be due to either enzyme inactivation or a change in the permeability of the muscle tissue to oxygen.

A microsomal fraction has been isolated from the muscle tissue of lean (McDonald et al., 1979) and fatty fish (Slabyj and Hultin, 1982) which contains an enzymic system capable of catalyzing the oxidation of the lipids of the membrane in vitro. The possible role of this membrane fraction in the oxidation of lipid in fish tissue, however, is not known. The objective of the present study was to determine whether a peroxidizing microsomal fraction from fish muscle could cause oxidation of a lipid emulsion in a model system.

MATERIALS & METHODS

HERRING (*Clupea harengus*) were harvested commercially off the coast of Maine and held in refrigerated sea water while in transit to the processing plant which took approximately 4 hr. At the plant, large fish (25-35 cm) were removed and placed in ice for transport to the laboratory. Subsequently, 4 to 5 fish were placed in plastic bags, double wrapped, frozen in a blast freezer at $-34°C$, and stored at $-90°C$. As needed a package of frozen herring was thawed in cold running water (1.5-2 hr), filleted, and microsomal fractions prepared from the light muscle tissue as previously described (Slabyj and Hultin, 1982). Frozen storage of herring at $-90°C$ had no effect

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on specific activity of lipid peroxidation of the membrane preparation. The protein concentration of each preparation was determined by the Lowry procedure as modified by Markwell et al. (1978).

Lipids were extracted from herring fillets of the same school of fish used for the preparation of microsomes by the method of Bligh and Dyer (1959). An emulsion was prepared by sonicating the lipid in water (25 mg of lipid per mL) for 15 sec with a Fisher Scientific Model 300 Sonic Dismembrator equipped with a microtip. This emulsion was added to the assay medium to give a final concentration of 2 mg of lipid per ml.

Microsomal lipid peroxidations were carried out in a medium containing 0.1 mM ADP, 0.1 mM NADH, 0.015 mM $FeCl_3$, and herring light muscle microsomes at a concentration of 0.04 mg of protein per mL in 0.12M KCl-5 mM hisitidine buffer at pH 7.3. Five mL volumes of assay system in 25 mL Erlenmeyer flasks were used to follow malondialdehyde (MDA) production and 50 mL volumes in 300 mL flasks for the purpose of analyzing the fatty acid composition of the microsomal fraction. The flasks were incubated in a gyratory temperature-controlled water bath at 6°C in duplicate. The reaction was initiated by adding an appropriate volume of the microsomal fraction.

Thiobarbituric acid reactive substances, expressed as MDA, were determined according to Buege and Aust (1978) using 1 mL frac-
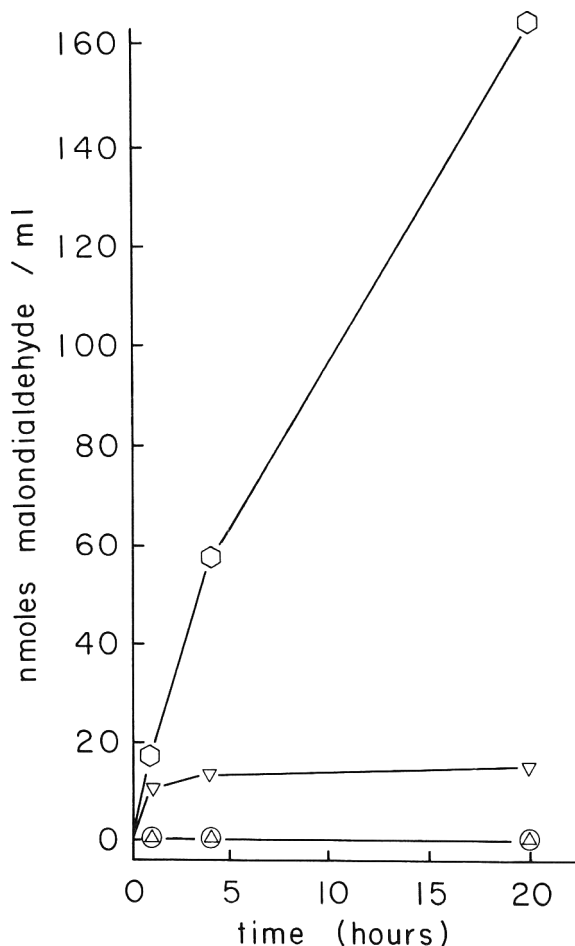

Fig. 1—Peroxidation of lipid emulsion by herring light muscle microsome fraction. Complete assay system (○), no lipid emulsion (▽), no HADH (△), and no microsomes (○).

tions to indicate degree of lipid oxidation. One-half mL of 1% butylated hydroxytoluene in absolute ethanol was added to 50 mL of the assay suspension (see above) to inhibit oxidation and the suspension was then centrifuged for 1 hr at 206,000 x g to harvest the microsomal fraction for fatty acid analysis. The microsomal fraction was then extracted with chloroform:methanol according to Bligh and Dyer (1959) and the extracted lipid transesterified with BF$_3$ (Ackman and Eaton, 1971) and analyzed on a Perkin-Elmer Model 3920B gas chromatograph, using a 2 mm x 1.8m glass column with 3% ethylenesuccinate-methylsilicone co-polymer on Gas Chrom P 100/120 mesh (Supelco) (Mai and Kinsella, 1979).

RESULTS & DISCUSSION

TYPICAL RESULTS are shown in Fig. 1. When the enzyme-catalyzed oxidation of the microsomal lipids was carried out in the absence of the lipid emulsion, MDA production leveled off at approximately 4 hr. After 20 hr a total of 14 nmoles of MDA were detected per mL of the assay medium. The amount of MDA produced per mL was noticeably greater in the presence of both the peroxidizing microsomal fraction and the lipid emulsion (the complete assay system), reaching 165 nmoles MDA per mL at the 20 hr sampling. These data clearly indicate that the difference in the amount of MDA produced between the two test runs was due to the use of the lipid emulsion.

When the microsomal fraction was deleted from the complete assay system, there was no production of MDA observed, indicating that the lipid emulsion was not autoxidized in the presence of NADH, ADP and iron. When NADH was deleted from the complete assay system, there was also no oxidation detected, indicating that nothing in the membrane preparation caused oxidation of the lipids in the emulsion. Since there was no oxidation in the system when the microsomal fraction was not undergoing the enzyme-catalyzed oxidation of the microsomal lipids, it must be assumed that somehow the peroxidation of the microsomal lipids initiated the oxidation of the lipids in the emulsion.

Production of TBA-reactive substances by the peroxidizing microsomal fraction in the absence of emulsion reached a plateau after approximately 4 hr. This corresponded to a reduction in the C20:5ω3 fatty acid from 12% of the total of the unoxidized microsomes to 0.2%, and in the 22:6ω3 fatty acid from 31% of the untreated microsomes to 0.1%. Eicosapentaenoic and docosahexaenoic acids in the complete assay system (peroxidizing microsomes in the presence of

emulsion), on the other hand, were reduced from 6 and 8% before the reaction was initiated to 3 and 2%, respectively, after 20 hr of incubation. The difference in eicosapentaenoic and docosahexaenoic concentration in the microsomal fraction and the complete assay system is due to the low concentration of these fatty acids in the lipid emulsion. The above observed change in fatty acid concentration would imply that in the assay system where lipid emulsion was not included, most of the available substrate for oxidation was exhausted within 4 hr, thus little additional production of MDA was possible. In the complete assay system it would appear that the microsomal fraction initiates peroxidation of the lipid emulsion. This study is, therefore, the first report in the literature to show that microsomal fractions obtained from herring tissue will initiate peroxidation of the lipid emulsion in a model system.

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Role of Fungi on Oil Quality of Stored Seeds of Sesame, Rape and Linseed

G. C. MONDAL and B. NANDI

──────────── ABSTRACT ────────────

Deteriorative efficacy of storage fungi through change in the quality of different edible oils was studied. Maximum loss of oil in both rape and linseed was recorded with *A. fumigatus* and in black cultivar of sesame with *A. niger*. Refractive indices of oil decreased in most of the cases with concomitant increase in free fatty acids (FFA). The deteriorated oil samples showed change in color, saponification value and iodine value with longer incubation which depended partly on the fungus involved and partly on the type of substrate. Both *A. niger* and *A. fumigatus* produced higher amount of lipase than others. Production of lipase enzyme and mycelia were always higher on emulsified oil than on seedmeal media.

INTRODUCTION

OILSEED LIKE RAPE, black cultivar of sesame and linseed are extensively cultivated and stored in different parts of the world including India, as their oils are used in culinary as well as for industrial purposes. These oilseeds often become invaded by fungi in both natural storage and under controlled conditions (Mondal et al., 1981; Nandi et al., 1982). Different species of these fungi produce varied amounts of lipase (Goodman and Christensen, 1952; Jensen, 1974; Chander et al., 1977) which change considerably the amount and quality of oil (Bose and Nandi, 1982). Consequently, not only is oil from the deteriorated seeds of inferior quality but also the cost for refining the oil rises from a normal 1-1.5% to over 5%, resulting in great economic loss to both farmers and the industry.

It was, thus deemed necessary to study the extent of deterioration in quality of oil by fungi in oilseed in storage and on the isolated oil itself.

MATERIALS & METHODS

Seeds

Healthy, freshly harvested seeds (70g) of black cultivar of sesame, rape and linseed were autoclaved for 20 min at 12 psi and the moisture content of the seeds were adjusted to 20% (dry weight basis) by adding a requisite amount of sterile deionized water.

Fungi

Seeds were inoculated separately with 0.5 mL spore suspension (6×10^5 spores/mL) prepared from 9 day old cultures of *Aspergillus fumigatus, A. niger, A. chevalieri,* and *A. funiculosus* predominantly associated with these oilseeds in natural storage. The inoculated seeds were thoroughly mixed for 2 days and incubated at 30 ± 2°C for 15 and 30 days. Identical sets of autoclaved but uninoculated seeds served as control.

Physical and chemical characteristics of oil

Oil was extracted from dried and ground seeds with petroleum ether (b.p. 40-60°C) in a soxhlet extractor for 6 hr (Meara, 1955). Physical characteristics like color intensity of the oil was measured with a Lovibond tintometer (Meara, 1955) and the refractive index

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of the oil sample was determined by a Bausch and Lomb Abbe 3L refractometer (AOAC, 1980). Chemical characteristics like free fatty acid (FFA) and saponification value of the oil were estimated following AOAC (1980) procedures. Iodine value of the oil was also determined following Tom's method described by Meara (1955).

Lipase production

The test fungi were grown on Czapek's Dox medium supplemented with high quality seed oil (5%) as the sole carbon source and on seed meal medium containing about 5% oil. After 15, 30 and 45 days of incubation, dry weight of mycelia of the test fungi and lipase activity were measured following Sharma and Chauhan (1976).

RESULTS & DISCUSSION

A MARKED REDUCTION in oil from the initial value was observed in fungus-inoculated seeds (Table 1). Rate of reduction was maximum when the seeds were inoculated with *A. fumigatus* and *A. niger* indicating thereby that these fungi were more efficient in decreasing oil content than *A. funiculosus* and *A. chevalieri*. Bose and Nandi (1982) reported loss of oil content to depend not only on the fungal species involved but also on the composition of fatty substrate.

All the test fungi resulted in a more evident increase of red and yellow units in the oil color during storage (Table 1). Except for *A. fumigatus*, none of the other species produced blue units. Such increased production of darker tints suggested synthesis of pigments in mycelium of the invading fungi (Robertson et al., 1973; Farag et al., 1981)

Refractive indices of oil from fungal deteriorated seeds were less than the control in all cases (except sesame) with the minimum in seeds inoculated with *A. niger* (Table 1) Such reduction might be due to formation of FFA in oil (Meara, 1955). In sesame, however, it increased in all cases with the maximum caused by *A. fumigatus* at the first phase of incubation followed by a slight decrease. An increase in refractive index of deteriorated sesame oil seemed to reflect an increase in the saturated acids (Milner, 1950)

FFA of oil from fungus inoculated seeds, which rose gradually during incubation (Table 1), indicated break down of oil by fungal lipase. The highest increase was noted when seeds were inoculated with *A. niger* and least with *A. fumigatus* in all oils except that of rape where minimum production was observed with *A. chevalieri*. Such variation in production of FFA might reflect the differences in lipase producing efficiency of the invading fungi. However, no linear correlation was evident between reduction of oil and increase of FFA which might be due to differences in the rate of oil degradation and subsequent consumption of FFA by the fungi (Wilson, 1947; Heaton et al., 1978).

The saponification value increased gradually with concomitant increase of FFA in deteriorated oil (Table 1). Increase in the saponification value reflected an increase in fatty acids and short chain glycerides during the lipolysis of oil. *A. niger* showed maximum increase in saponification value of oil except in rape where it was induced by *A. funiculosus*, although it produced lower FFA indicating utilization of a higher amount of FFA as carbon source.

A marked decrease in iodine value of oil deteriorated by the test fungi (Table 1) suggested either selective utilization

Table 1—Changes in the physico-chemical properties of oil by predominant seed storage fungi.

Seed	Fungi	Oil content (%) Incubation (days) 0	15	30	Color intensity (Units)[a] Incubation (days) 0	15	30	Refractive index (at 40°C)[a] Incubation days 0	15	30	Fat acidity value[a] Incubation (days) 0	15	30	Saponification value[a] Incubation (days) 0	15	30	Iodine value[a] Incubation (days) 0	15	30
Rape	Uninoculated (control)	40.0	40.2	40.0	20Y 0.8R	20Y 0.8R	20Y 0.8R	1.4637	1.4637	1.4637	5.21	8.84	10.2	171.0	171.8	172.0	101.5	100.5	99.7
	A. fumigatus		36.3	32.8		30Y 1R	30Y 1R 0.3B		1.4632	1.4622		75.7	234.8		180.7	193.6		83.6	53.7
	A. niger		36.0	33.2		40Y 3R	30Y 3R		1.4612	1.4602		509.7	515.7		180.6	186.3		74.7	59.0
	A. chevalieri		36.0	35.5		30Y 2R	40Y 1R		1.4627	1.4622		74.1	128.0		184.0	188.2		61.6	61.0
	A. funiculosus		36.2	36.0		30Y 3R	40Y 5R		1.4622	1.4617		147.6	292.3		189.0	194.2		79.5	70.4
Sesame	Control	46.0	45.7	45.9	1Y 0.3R	1Y 0.3R	1Y 0.3R	1.4611	1.4611	1.4611	3.0	7.5	8.9	185.0	185.7	186.6	116.0	118.0	121.0
	A. fumigatus		38.6	32.5		1.4Y 0.2R	2Y 0.6R 0.3B		1.4655	1.4652		130.4	165.7		193.2	215.0		77.6	73.5
	A. niger		38.0	32.0		20Y 1R	20Y 2R		1.4641	1.4635		317.0	580.6		192.0	219.0		90.0	93.1
	A. chevalieri		44.0	40.0		10Y 0.6R	20Y 0.4R		1.4651	1.4640		134.6	212.0		189.0	193.0		75.5	71.2
	A. funiculosus		45.0	41.0		20Y 0.1R	20Y 0.3R		1.4645	1.4642		176.7	188.5		187.4	203.0		90.0	74.5
Linseed	Control	37.0	36.7	37.2	15Y 0.6R	15Y 0.6R	15Y 0.6R	1.4726	1.4725	1.4726	2.8	3.7	4.9	191.0	191.9	191.8	175.0	176.5	178.0
	A. fumigatus		32.0	29.7		12Y 1R	11Y 1R 0.4B		1.4720	1.4712		75.7	126.3		210.0	224.2		171.0	165.0
	A. niger		32.6	31.0		20Y 1R	11Y 1R		1.4691	1.4717		420.8	426.0		193.0	223.9		209.0	180.0
	A. chevalieri		35.5	35.5		30Y 0.9R	40Y 1R		1.4717	1.4713		117.8	134.7		201.0	205.0		172.2	170.0
	A. funiculosus		33.0	32.6		15Y 2R	30Y 2R		1.4711	1.4710		210.4	224.5		196.3	217.0		166.0	159.2

[a] Mean value of three replicates; Y = Yellow; R = Red; B = Blue.

Table 2—Lipolytic activity of predominant seed storage fungi on different oil emulsified and seedmeal media

Seed	Fungi Incubation (days)	Mycelial dry matter (mg)/50 mL medium[c] a[a] Media 15	30	45	b[b] 15	30	45	Lipase activity (units)/50 mL culture[c] a[a] Media 15	30	45	b[b] 15	30	45
Rape	A. fumigatus	122	135	110	102	121	103	2.0	5.4	1.5	4.0	3.2	2.5
	A. niger	231	267	279	130	161	178	30.7	45.6	21.5	9.5	5.0	2.5
	A. chevalieri	122	128	117	110	121	109	2.1	3.1	1.0	2.0	1.5	0.5
	A. funiculosus	142	176	168	121	139	113	1.9	3.4	2.0	3.0	2.5	1.7
	Uninoculated (control)							0.2	0.1	0.1	0.3	0.1	0.2
Sesame	A. fumigatus	125	140	122	105	122	95	2.5	5.2	2.0	4.5	2.3	1.2
	A. niger	250	280	295	210	235	242	39.0	56.0	27.0	7.5	5.0	2.7
	A. chevalieri	140	185	128	116	128	102	4.5	5.0	2.5	3.5	2.0	0.9
	A. funiculosus	180	226	215	124	138	112	2.0	3.5	1.5	3.5	2.0	0.9
	Uninoculated (control)							0.2	0.1	0.2	0.2	0.1	0.3
Linseed	A. fumigatus	132	148	125	118	127	110	3.5	6.7	3.0	3.6	1.5	1.5
	A. niger	225	252	267	135	198	220	32.5	34.0	18.5	12.0	3.9	2.0
	A. chevalieri	135	168	138	102	126	95	2.6	3.7	1.5	1.4	0.8	0.8
	A. funiculosus	145	190	152	112	130	105	2.1	4.1	1.8	1.5	0.9	0.8
	Uninoculated (control)							0.2	0.2	0.1	0.3	0.2	0.1

[a] a = Czapek's Dox emulsified oil medium.
[b] b = seed meal medium.
[c] Mean value of three replicates.

of some unsaturated acids as a carbon source or to oxidaion of oil with the formation of oxy-acids. An increase in iodine value of linseed oil which was induced by A. niger reflected higher unsaturation in the fatty acids synthesized by metabolic activity of the fungus that was responsible for changing a part of the saturated oleic and linoleic acids.

A. niger and A. fumigatus produced higher lipase units on both media than others indicating thereby that these species could utilize more successfully the supplemented vegetable oils of the test seeds in the media as a carbon source (Table 2). Maximum lipase production by A. niger on sesame oil and by A. fumigatus on linseed oil suggested the presence of some specific esters in the media that might have stimulated higher production. A comparatively higher rate of lipase production at the first phase might be responsible for more rapid breakdown of oil into FFA which

were then utilized as carbon source as was evident from higher mycelial dry matter of each fungus (Table 2).

Thus, the test fungi proved to be very efficient in the utilization of complex fatty substances by secreting extracellular lipases thereby causing extensive damage in quality of oil in stored seeds which ulimately became unfit for industrial use or direct consumption for edible purposes.

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Composition of Flours from Samoan Breadfruit

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————————ABSTRACT————————

Flours from the pulp of seven varieties of breadfruit (*Artocarpus altilis*) at various stages of maturity were prepared and analyzed. Crude protein content ranged from 2.90–5.08%, crude fat from 0.80–1.93%, crude fiber from 2.87–6.56%, ash from 1.90–4.08%, total sugars from 10.0–31.8%, and starch from 53.4–75.7% depending on variety and stage of maturity. Fructose was the predominant sugar in less mature fruits, becoming less so in comparison to glucose and sucrose as maturation progressed. The proportion of unsaturated to total fatty acids in the lipid component varied between varieties from 41-64%. Observed levels of iron, sodium, phosphorus, calcium, and potassium may be significant in a nutritional sense.

INTRODUCTION

THE BREADFRUIT TREE is grown widely in tropical areas where it is an important food source. It is known by the botanical names *Artocarpus communis*, *Artocarpus incisus*, and *Artocarpus altilis* with the last of these being most widely accepted (Stone, 1974). Despite its ease of cultivation in the tropics, breadfruit appears to be under-exploited as a food principally because of its poor post-harvest storage behavior. Nevertheless conversion of the fruit into a stable form such as a dry flour offers a solution to storage stability problems.

Utilization of such a product depends largely on the development of food products based on the flour and perhaps isolation of suitable components, especially starch, for other uses. Data on the composition of the breadfruit are of obvious importance in this regard. Recently Graham and De Bravo (1981) examined changes in composition of Puerto Rican breadfruit. Starch from a similar source was studied by Loos et al. (1981).

In common with many tropical regions, breadfruit are cultivated and utilized in Samoa where over 30 varieties are recognized. These varieties are distinguished primarily by the presence or absence of seeds in the fruit, the shape and skin texture of the fruit and the leaf structure. In addition to these features breadfruit varieties also differ in season of maturity, cooking quality and flavor. Thus it is likely that differences in chemical composition also exist between varieties which in turn would affect nutritional quality and suitability for processing. The purpose of this study was to analyze flours prepared from the pulp (edible portion) of seven Samoan breadfruit varieties at various stages of maturation to provide data which would assist in selection of varieties most suitable for widespread cultivation.

MATERIALS & METHODS

BREADFRUITS were harvested from seven different cultivars at the very immature, immature, mature and very mature stages where possible. The appearance of the fruit at each stage was similar to that described by Graham and De Bravo (1981). For each variety the samples were collected during October-December, 1980, from the same trees which were grown at the Agricultural Research Sta-

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tion at Mafanua, Western Samoa. Because of differences in rate and season of maturation it was not possible to harvest all four maturity stages for all varieties.

Preparation of breadfruit flour

The breadfruits were processed immediately after harvest as follows: The fruits were hand peeled, decored and the pulp sliced lengthwise into eight equal parts prior to soaking for 30 min in 5% sodium metabisulphite solution to prevent enzymic browning. These parts were then cut into chips of 5 mm thickness using a potato chipper. The chips were dried on stainless steel trays in an air drier at 40°C to a moisture content of approximately 10%. The dried product was milled into a coarse flour in a hammer mill and sieved through a 1 mm sieve to give the finished breadfruit flour.

Chemicals and reagents

All chemicals and reagents were of analytical grade.

Analytical methods

Protein (N × 5.70), fat, fibre, moisture, ash and starch were determined using procedures outlined in the AOAC (1975). Individual and total sugars were determined by HPLC analysis of an 85% methanolic extract of the flours using a Waters Associates carbohydrate column with acetonitrile/water (85/15) as mobile phase; identity and quantitation were achieved by injection of standard solutions of various sugars. Individual metals were determined by Atomic Absorption Spectrometry after samples were ashed in the presence of hydrochloric acid. Phosphorus was determined by the method of Morrison (1964). Fatty acid composition was established by gas-liquid chromatography of methyl esters prepared from a chloroform extract of flour lipids. Starch was isolated as described by Loos et al. (1981) and amylose levels were measured using the method of Williams et al. (1970).

RESULTS & DISCUSSION

THE PROXIMATE COMPOSITION of flours prepared from the pulp of seven Western Samoan breadfruit varieties at different stages of maturity is shown in Table 1. Moisture content varied considerably between flour samples, and compositional data are presented on a dry weight basis to allow easier comparison of the results. Considering first the mature stage, that preferred for harvest and consumption, there are obvious and potentially important differences between varieties in terms of protein and starch levels.

The varieties *Ulu puou* and *Ulu talatala* offer significantly higher protein levels than the others (4.46% and 5.00%, respectively) with *Ulu maa* (2.90%) being poorest in this regard. In terms of starch content, *Ulu maopo*, *Ulu puou* and *Ulu maa* all yielded a flour with over 70% starch content. Total sugar contents varied considerably with *Ulu maafala* and *Ulu talatala* having almost twice the sugar content of *Ulu puou* (11.5%). Crude fat varied from 0.80–1.93%, crude fiber from 2.87–5.01% and ash from 1.90–3.18% among varieties at the mature stage. All values obtained were comparable with those obtained by Graham and De Bravo (1981) for flour from Puerto Rican breadfruit pulp.

These data are of obvious importance in the selection of breadfruit varieties for different end uses where requirements may vary in a compositional sense. For example *Ulu talatala* has a higher protein content than most varieties

Table 1—Composition of flour from pulp of breadfruit at different stages of maturity

| Variety | Maturation stage | Moisture content (%) | % (dry weight basis) of: | | | | | |
			Crude protein	Crude fat	Crude fibre	Ash	Total sugar	Starch
Ulu maopo	Immature	8.60	4.60	1.33	6.56	2.99	15.1	66.7
	Mature	6.17	3.34	1.93	5.01	2.55	15.6	71.3
	Very mature	4.83	4.34	1.57	4.73	3.08	31.5	53.4
Ulu puou	Very immature	11.22	4.72	0.77	4.70	3.09	19.7	65.4
	Immature	11.65	4.76	0.80	3.83	2.94	14.8	72.1
	Mature	13.73	4.46	0.96	3.75	2.84	11.5	73.1
Ulu maafala	Immature	3.61	3.57	1.11	3.20	1.69	2.17	66.2
	Mature	2.84	3.70	1.35	2.87	1.90	22.1	66.7
	Very mature	2.55	4.00	1.25	2.84	2.41	31.8	56.1
Ulu talatala	Immature	21.02	4.77	1.29	6.86	4.08	18.6	62.7
	Mature	19.03	5.00	1.35	5.27	2.48	22.7	61.5
	Very mature	15.45	5.08	1.45	5.19	2.59	27.9	53.9
Ulu gutufagu	Immature	14.42	4.52	0.84	4.67	3.54	10.0	75.7
	Very mature	14.91	4.00	0.80	4.15	3.91	14.6	67.9
Ulu maa	Immature	3.40	3.79	1.25	4.14	2.86	14.8	70.7
	Mature	8.84	2.90	0.80	4.12	3.18	17.0	71.4
Ulu aveloloa	Mature	9.79	3.26	1.66	4.49	2.54	18.7	67.7

Table 2—Levels of fructose, glucose and sucrose in flour from the pulp of breadfruit at different stages of maturity

| Variety | Maturity stage | % (dry weight basis) of: | | |
		Fructose	Glucose	Sucrose
Ulu maopo	Immature	10.4	2.9	1.8
	Mature	9.0	3.6	3.0
	Very mature	8.5	11.3	11.7
Ulu puou	Very immature	13.2	6.5	—*
	Immature	9.9	4.9	—
	Mature	4.8	6.4	0.4
	Very mature	4.3	8.7	1.3
Ulu maafala	Immature	10.2	4.6	6.9
	Mature	9.5	5.0	7.5
	Very mature	11.1	4.3	16.4
Ulu talatala	Immature	10.4	8.2	—
	Mature	12.9	9.7	—
	Very mature	13.6	8.8	5.9
Ulu gutufagu	Immature	7.3	2.7	—
	Very mature	7.9	6.7	—
Ulu maa	Immature	5.0	2.7	7.1
	Mature	4.3	3.1	9.5
Ulu aveloloa	Mature	6.4	2.9	9.0

* <0.1%

and hence may be nutritionally superior. However, it is least promising in terms of potential starch yield. Starch yield is not the only criterion for selection in this context. Although starches from each variety were similar by microscopic examination to that isolated by Loos et al. (1981), amylose levels varied with *Ulu puou* starch being lower in this regard (16.4%) than others examined in this study (18.6–19.8%) and that previously observed (18.2%) by Loos et al. (1981). This property may have considerable practical importance in utilization of breadfruit starch. The large variations in total sugars are also of potential importance in the production of fermented breadfruit products such as "masi-ulu," a fermented breadfruit paste which has been discussed by Cox (1980).

There is no clear pattern of compositional change within varieties during maturation save for a decrease in starch and increase in total sugars between the mature and very mature stages. This increase in sugar level is thought to be part of

the reason why very mature breadfruit are generally not used as food because of greater sweetness, although mature *Ulu maafala* and *Ulu talatala*, varieties highest in sugar, are quite acceptable in Western Samoa. Because of the large changes in sugar level during maturation, its wide variations between varieties and its apparent importance in acceptability, changes in individual sugars during maturation were studied. Fructose, glucose and sucrose were the major sugars present with ribose and maltose being detectable in trace amounts (<0.1%). These last two were not quantified. Shown in Table 2 are the levels of fructose, glucose and sucrose found in flour prepared from the pulp of the seven breadfruit varieties at different stages of maturity. In less mature fruit, fructose was present in higher levels than glucose which increased proportionally with the age of the fruit. Sucrose levels also increased as the fruit matured especially between the mature and very mature stages. The magnitude of these changes, especially increases in sucrose, correlates with starch breakdown (Table 1). Differences in total sugars between flours prepared from mature breadfruit were not attributable to any particular sugar.

Although present in low levels (crude fat 0.80–1.93%), the fatty acid composition of breadfruit flour lipids was determined for each variety because of the possibility of fat rancidity and hence poor storage life and/or acceptability of the product. The major fatty acids present were myristic, palmitic, stearic, oleic, linoleic and linolenic. Unsaturated acids represented between 41–64% of total fatty acids with linolenic acid ranging from 13–30%, depending on variety. It should be noted that rancidity development was not apparent in any of the flours after storage for 6 months at 5°C.

Levels of iron, sodium and calcium found in flour from the pulp of mature breadfruit varieties were similar to those observed by Graham and De Bravo (1981) although potassium was approximately half and phosphorous four times in the present work.

This research has shown the variation in composition of breadfruit flour which can result from varietal effects. This is particularly important for levels of sugars, protein and starch in terms of end use of the product and should aid in selection of varieties most suitable for a given purpose. It is also obvious that more basic research is required to elucidate fully the properties of breadfruit starch, the amino

Identification of 3,5-Diisobutyl-1,2,4-trithiolane and 2-Isobutyl-3,5-diisopropylpyridine in Fried Chicken Flavor

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————————————ABSTRACT————————————

3,5-Diisobutyl-1,2,4-trithiolane and 2-isobutyl-3,5-diisopropylpyridine were identified in the volatiles isolated from fried chicken. The identifications were confirmed by organic synthesis. Mechanisms for the formation of these two compounds in fried chicken flavor are discussed.

INTRODUCTION

A RECENT INVESTIGATION in our laboratory reported the identification of 130 compounds in fried chicken flavor (Tang et al., 1983). Two interesting mass spectra which remained unidentified were believed to be a pyridine (MW 219) and a trithiolane (MW 236). Subsequent study led to the proposal that these compounds were 2-isobutyl-3,5-diisopropylpyridine and 3,5-diisobutyl-1,2,4-trithiolane. Neither of these compounds has been reported in natural food flavors. However, both have been reported in model system studies (Shu et al., 1981; Hartman et al., 1983).

This communication reports the synthesis and identification of these two compounds in order to confirm their presence in fried chicken flavor

MATERIALS & METHODS

Preparation of 3,5-Diisobutyl-1,2,4-trithiolane

3,5-Diisobutyl-1,2,4-trithiolane was prepared by modifying the method of Shu et al. (1981). Isovaleraldehyde was mixed with aqueous 22% ammonium sulfide in a 1:1 molar ratio at room tem-

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perature for two hours. The reaction mixture was extracted with diethyl ether. The ether extract obtained was washed with water until neutral, dried over anhydrous sodium sulfate and concentrated.

Preparation of 2-Isobutyl-3,5-diisopropylpyridine

2-Isobutyl-3,5-diisopropylpyridine was prepared by reaction of 23 mL. (0.2 mole) of isovaleraldehyde with 100 mL of 58% ammonium hydroxide (0.1 mole of NH_3) at 200°C for 1 hr. The reaction product was washed four times with 100 mL portions of distilled water, dried over anhydrous sodium sulfate and vacuum-distilled (0.5 mm Hg).

Two fractions were obtained, bp. 39–52°C and 75–110°C. The first fraction was unreacted isovaleraldehyde (11.8g) and the second the reaction product (6.6g). 2-Isobutyl-3,5-diisopropylpyridine accounted for 68% (4.5g) of the product.

RESULTS & DISCUSSION

MASS SPECTRAL DATA obtained for synthetic 3,5-diisobutyl-1,2,4-trithiolane and 2-isobutyl-3,5-diisopropylpyridine appear in Table 1. The presence of these compounds in fried chicken flavor was confirmed by comparison with the unknown mass spectra.

3,5-Diisobutyl-1,2,4-trithiolane has been reported to possess roasted, roasted-nut, crisp bacon-like and pork rind-like aromas and flavors (Shu et al., 1981). The lower homolog of this compound, 3,5-dimethyl-1,2,4-trithiolane, has been reported in boiled beef (Chang et al., 1968) and fried chicken flavor (Tang et al., 1983). 3,5-Diisobutyl-1,2,4-trithiolane has been produced in a model system containing isovaleraldehyde, ammonia and hydrogen sulfide (Shu et al., 1981). Isovaleraldehyde may arise via Strecker degradation of leucine. Ammonia and hydrogen sulfide may arise via thermal degradation of amino acids and sulfur-containing amino acids, respectively.

2-Isobutyl-3,5-diisopropylpyridine was found to possess a roasted cocoa-like aroma. The formation of pyridines from reaction of aldehydes and ammonia (Chichibabin condensation) in foods and model systems has been re-

Table 1—3,5-Diisobutyl-1,2,4-trithiolane and 2-isobutyl-3,5-diisopropylpyridine identified in fried chicken flavor

	Compounds Isolated from fried chicken flavor; MS data[a], m/z (%)	Reference Compound MS data[a], m/z (%)
3,5-diisobutyl-1,2,4-trithiolane	41(84), 43(62), 55(22), 60(22), 69(100), 73(18), 85(12), 87(26), 101(22), 102(34), 115(12), 129(20), 134(12), 171(12), 172(10), 203(3), 220(2), 236(22), 237(3)	41(75), 43(70), 55(25), 60(32), 69(100), 73(20), 85(12), 87(28), 101(35), 102(35), 115(10), 129(30), 134(10), 171(10), 172(8), 203(2), 220(3), 236(30), 237(3); M = 236
2-isobutyl-3,5-diisopropylpyridine	41(22), 43(18), 55(15), 57(10), 67(6), 69(14), 82(9), 83(16), 91(9), 97(10), 109(50), 111(7), 119(4), 120(7), 134(7), 135(15), 149(100), 150(14), 162(72), 163(11), 176(27), 177(49), 188(9), 204(69), 205(14), 218(15), 219(37)	41(35), 43(20), 55(20), 57(8), 67(5), 69(15), 82(10), 83(15), 91(10), 97(15), 109(7), 111(9), 119(6), 120(8), 134(7), 135(20), 149(100), 150(10), 162(60), 163(8), 176(20), 177(22), 188(10), 204(50), 205(6), 218(10), 219(35); M = 219

[a] The two most intense ions every 14 mass units above 20 are listed.

A Research Note
Behavior of Phospholipids in Soybean Oil on Silicic Acid

A. P. HANDEL and D. D. WINTERS

─────── ABSTRACT ───────

The unexpected recovery of soybean oil phospholipids in the nonpolar eluents from a silicic acid column led to this investigation. Crude soybean oil dissolved in ethyl ether was mixed with silicic acid, filtered and the amount of phosphorus determined in the filtrate. Increasing time of contact with silicic acid, and pretreatment by heat or sonication decreased the amount of phosphorus in the filtrate. These findings indicate that phospholipids in crude soybean oil occur in a form that gives them a nonpolar character that can be changed. However, a certain portion of the phospholipids are not changed by the treatments described. These results are compared to phosphorus loss by degumming of soybean oil.

INTRODUCTION

THE CLASSIC PROCEDURE of Hirsch and Ahrens (1958) to separate lipid mixtures uses solvents of increasing polarity with columns of silicic acid. In their procedure, phospholipids are eluted from a column with methanol following elution of the other lipid classes. In applying this elution scheme to crude soybean oil it was found that only a portion of the total phosphorus in a sample was recovered in the phospholipid fraction (Racicot and Handel, 1980). Part of the total phosphorus eluted with the neutral lipids prior to the addition of methanol. As a result, in analysis of individual phospholipids of crude and degummed oil, 4% ethyl ether in petroleum ether was used to remove neutral lipids without loss of phosphorus in the neutral fraction (Racicot and Handel, 1983). Another part of the total phosphorus can be attributed to phytic acid which does not elute from the column (Winters et al., 1984).

Kito et al. (1979) have described a protein-phospholipid complex in the form of an inverted micelle that has nonpolar surface properties. This might account for the elution of phosphorous containing compounds in neutral fractions. The association of phospholipids with cations such as calcium and magnesium could also influence their behavior on silicic acid. Brown and Snyder (1984) have reported phospholipids are irreversibly adsorbed on silica. This study was designed to test the effect of contact time, heat, sonication and degumming on the interaction of phospholipids with silicic acid.

MATERIALS & METHODS

CRUDE SOYBEAN OIL was obtained from a commercial soybean refinery in Lincoln, NE. In order to disrupt possible micellar structures, some aliquots of crude oil were pretreated by sonication for 1 hr or by heat at 100°C for 1 hr.

Slurry procedure

One gram of crude soybean oil was dissolved in 100 mL diethyl ether. Added to this were 25g Bio-Sil A (100-200 mesh) (Bio Rad Laboratories, Richmond, CA) that had been activated for at least 1 hr at 110°C. A magnetic bar stirred this slurry vigorously for 5, 15,

30 or 60 min in a 250 mL covered beaker. Heated and sonicated oils were both stirred for 15 min. The slurry was then transferred to a 150 mL Buchner funnel with a coarse porosity fritted glass disk and then vacuum filtered. The silicic acid was washed with an additional 50 mL of diethyl ether. Solvent was removed using a rotary evaporator and the sample was brought to 10 mL with diethyl ether in a volumetric flask prior to determination of phosphorus.

Degumming

Degumming was performed by stirring 147.25g crude oil and 2.75g water at 400 rpm and 60°C, according to the method of Al-Kahtani et al. (1984). Following centrifugation, phosphorous content was determined on the degummed oil and the degumming efficiency determined as the percentage phosphorus removed by degumming. A portion of the degummed oil was then treated by the slurry procedure, stirring for 60 min.

Phosphorus determination

Aliquots of the ether fraction from the slurry procedure were spotted on HPTLC plates and then charred and scraped according to the method of Racicot and Handel (1983). The scraped spots were then digested and analyzed for phosphorous according to the procedure of Rouser et al. (1966) using half amounts of reagents.

RESULTS & DISCUSSION

THE RESULTS of the slurry procedure are given in Table 1. The original sample of crude oil contained 702 μg phosphorus/g oil. The amount of phosphorus in the ether fraction decreased with increasing length of stirring time. This suggests that contact with silicic acid can, over time, change the form of the phospholipids in soybean oil so they are retained on the oclumn. The ether fraction of the oils pretreated by sonication or heat and stirred for 15 min contained slightly less phosphorus than the nonpretreated oils stirred for 60 min, and about one-fourth of the phosphorus in the ether fraction of nonpretreated oils stirred for 15 min. This suggests sonication and heat treatments have the same effect on the phospholipids as 60 min of contact with silicic acid. These effects could include disruption of an inverted micelle or breaking of weak associative bonds that give the phospholipids a nonpolar character.

Table 1—Phosphorus remaining in ether fraction of the slurry procedure

Sample treatment	Phosphorus[a] (μg P/g oil)	% Phosphorus[b]
5 min	172.9 ± 16.3	24.7
15 min	139.3 ± 31.9	19.9
30 min	88.4 ± 12.1	12.4
60 min	46.5 ± 12.0	6.6
Sonication and 15 min stirring	40.4 ± 19.6	5.8
Heating and 15 min stirring	40.4 ± 18.3	5.8
Degumming and 60 min stirring	49.7 ± 3.3	7.1

[a] Average of duplicate determinations.
[b] Calculated as percentage of total phosphrus in the original sample.

The level of phosphorus in oil after degumming was 82.4 ± 17.3 µg P/g oil, which is about the same level as the ether fraction after 30 min of stirring. Stirring the degummed oil with silicic acid for 60 min reduced the phosphorus to the same level as untreated samples with 60 min of stirring, or pretreated samples with 15 min stirring. These results suggest there is a level of phosphorus (in this sample about 40 µg P/g of oil) that is not removable by these simple techniques. This agrees with the general theory that a certain portion of phospholipids in soybean oil is not hydratable (Hvolby, 1971). However, pretreatment with strong acid or acetic anhydride (Segers, 1982; Hayes and Wolff, 1956) can be effective in removing more phosphorus during degumming.

This study has some implications for researchers working with soybean oil phospholipids. First, the phospholipid fraction obtained by silicic acid column chromatography, although rich in phospholipids, may not represent all of the phospholipids present in the oil. Secondly, phospholipids added back to soybean oil to determine their effects on stability or other properties may not truly reflect the physical form of the native phospholipids.

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BREADFRUIT FLOUR COMPOSITION . . . From page 1397

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VOLATILES IN FRIED CHICKEN FLAVOR . . . From page 1398

viewed (Vernin, 1982). This compound has been produced in a model system containing glucose and leucine which possessed a cocoa-like aroma (Hartman et al., 1983).

In conclusion, 3,5-diisobutyl-1,2,4-trithiolane and 2-isobutyl-3,5-diisopropylpyridine have been confirmed as natural components of fried chicken flavor.

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Weast, R.C. (Ed.). 1981. "Handbook of Chemistry and Physics," 62nd ed. The Chemical Rubber Co., Cleveland, OH.

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Cardello, A.V. and Maller, O. 1982. Acceptability of water, selected beverages and foods as a function of serving temperature. J. Food Sci. 47: 1549.
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Takeguchi, C.A. 1982. Regulatory aspects of food irradiation. Paper No. 8, presented at 42nd Annual Meeting of Inst. of Food Technologists, Las Vegas, NV, June 22-25.

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Peleg, M. 1982. Unpublished data. Dept. of Food Engineering, Univ. of Massachusetts, Amherst.
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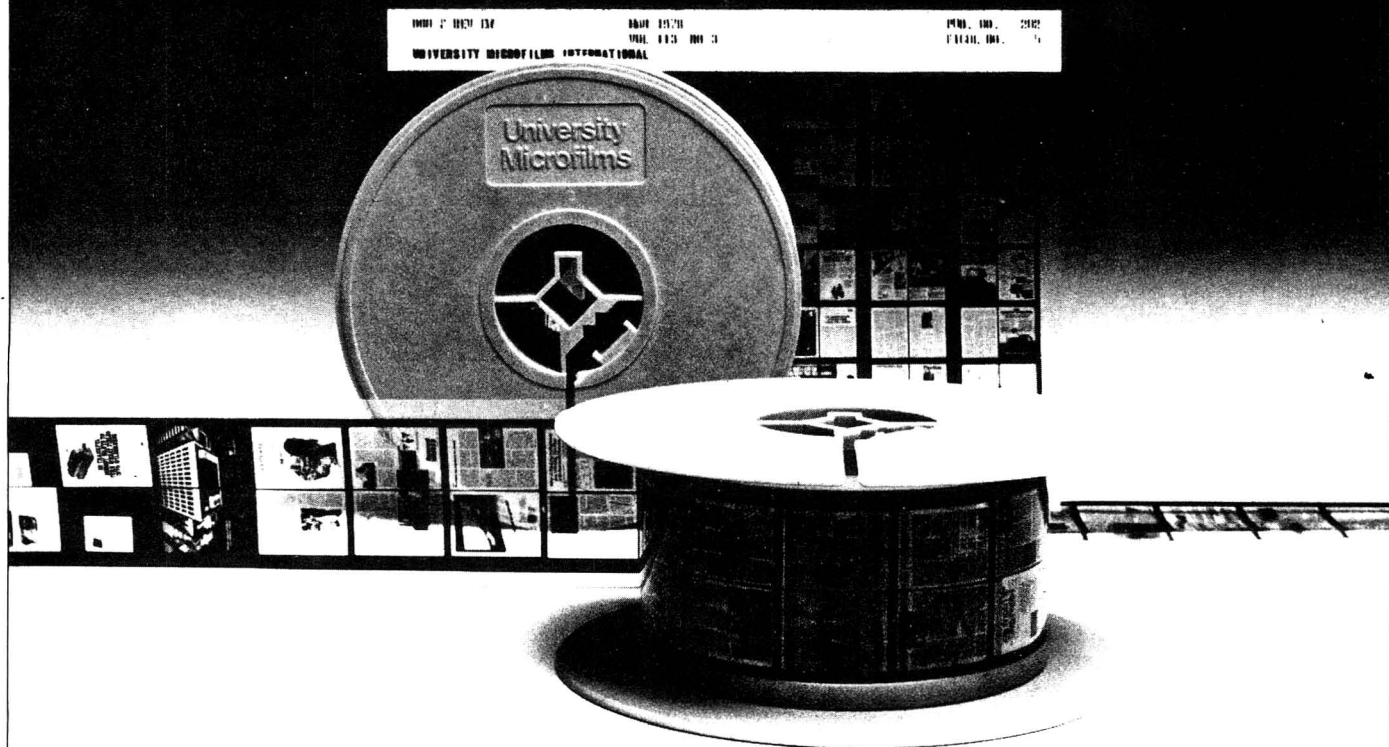
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