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PRODUCTION OF QUALITY QUICK-COOKING BEANS BY A COOKING/DEHYDRATION PROCESS

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ABSTRACT

Quick-cooking (4–5 min) beans of good organoleptic quality and low butterflying degree were made by blanching in boiling water to a water/bean (W/B) ratio of 0.8, cooking at 15 psig for 2 h, and drying with air at 100c and 700 fpm, tray depth of 5 cm, to 10% moisture. Bean flavor development in cooking increased with increasing W/B ratio, cooking pressure and time. Recooked dry bean quality increased with increasing air temperature and decreasing velocity and W/B ratio. Butterflying depended mostly on W/B ratio, decreasing sharply with decrease of the latter.

INTRODUCTION

Common beans (*Phaseolus vulgaris*) are a very popular food in Mexico and other developing countries. Together with maize tortillas, beans constitute the bases of the Mexican diet. Like other legumes, however, beans have a serious drawback: the long cooking time required. In the case of low-income families, a long cooking time becomes an economic burden, since substantial expenditures of fuel are required.

A number of attempts have been made with the purpose of developing quick-cooking beans (Steinkraus *et al.* 1964; Rockland and Matzler 1967; Bhaha *et al.*

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1967; Dorsey *et al.* 1961; Feldeberg *et al.* 1956). None, however, has resulted in dry beans of acceptable quality. Thus, the Steinkraus process, which is based on precooking followed by dehydration and requires addition of sugars to prevent bean butterflying during dehydration, would be of little value in Mexico, where sweet beans are totally unacceptable. The Rockland process, on the other hand, bestows quick-cooking properties as a result of treatment of beans with alkaline salts. The alkaline or low acid pH of the beans, however, markedly modifies color and flavor, again rendering them totally or partially unacceptable. The remaining processes are less well known, and are less practical than those of Steinkraus and Rockland.

The purpose of this research was to develop a simple process based on precooking followed by dehydration for the production of quick-cooking beans which would not have the limitations of the Steinkraus and Rockland processes. The general approach was to find processing conditions which would (1) obtain maximum flavor development during cooking; (2) obtain maximum flavor retention during dehydration; (3) reduce butterflying during dehydration; and (4) result in minimum cooking time for the dehydrated product.

MATERIALS AND METHODS

Thus, the first part of the research was a study of cooking variables. Cooking variables investigated were: (1) water/bean ratio, obtained by soaking beans in water; (2) soaking temperature; (3) cooking pressure (temperature); and (4) cooking time. A 2^4 factorial design (four independent variables with each at two levels; total number of experiments therefore equal to $2 \times 2 \times 2 \times 2 = 16$; Davies 1963) was selected as shown in Table 1. The general procedure employed in all experiments was as follows. Raw, dry beans were weighed and blanched 30 min by immersion in boiling water. The beans were allowed to soak in fresh water at a controlled temperature (as noted in Table 1). The soak water was discarded and the beans placed inside a pressure cooker and cooked at the required pressure for the required time; the pressure cooker was partially filled with water which

TABLE 1.
EXPERIMENTAL DESIGN (2^4 FACTORIAL) FOR THE COOKING RUNS

CODE	FACTOR	LOW LEVEL	HIGH LEVEL
A	WATER/BEAN RATIO	1.0	1.2
B	COOKING TIME	1 HR	2 HR
C	SOAK WATER TEMPERATURE	25°C	100°G
D	COOKING PRESSURE	10 PSIG	15 PSIG

was, however, not allowed to come into direct contact with the beans. After cooking, the beans were served to a fifteen-member nonexpert panel who were asked to rate the beans with respect to the following quality attributes on a scale of 0–10: (1) flavor; (2) color; (3) texture; (4) aroma; and (5) overall acceptance. Results of the experiments were analyzed by Analysis of Variance techniques (Davies 1963), utilizing triple and quadruple interactions as estimates of error. Separate analyses were performed for each of the quality attributes.

The second part of the research was the investigation of the drying process. Three separate studies were carried out each employing beans cooked as described to yield maximum organoleptic quality scores, with water/bean ratios of 0.8, 1.0 and 1.2, respectively. Although the 0.8 water/bean ratio was not considered in the cooking experiments, it was included in the drying experiments because butterflying during dehydration was believed to be sensitive to water/bean ratio and might be substantially decreased by reducing this ratio. Dehydration variables studied were: (1) air temperature (dry bulb); means for controlling the wet bulb temperature were not available; (2) air velocity; and (3) bean depth on tray. A factorial design, with the dehydration variables at two levels, and the water/bean ratio at three levels was employed (Davies 1963), as shown in Table 2. Beans were cooked according to the maximum organoleptic quality conditions determined in the cooking research and loaded onto the dryer trays at the required depth. The loaded trays were placed inside of a direct gas flame-heated dryer, and the beans dried with air at the required temperature and velocity, until 10% moisture had been attained (final bean weight was determined by calculation = 1.11Wo (1–0.01 Mo), where Wo and Mo are, respectively, initial weight and moisture content of beans loaded onto tray; initial bean moisture was determined according to AOAC, 1970 methods). Immediately after drying, percent butterflying was determined (in duplicate) by manually separating butterflied beans from a 100 g sample and weighing. The dry beans were cooked in boiling water until soft, thus measuring cooking times (bean softness was subjectively determined by one of the members of the research team, always the same person, by squishing 3 individual beans, taken at random, between his index finger and thumb; beans

TABLE 2.
EXPERIMENTAL DESIGN FOR THE DRYING RUNS

CODE	FACTOR	LOW LEVEL	INTERMEDIATE LEVEL	HIGH LEVEL
A	AIR VELOCITY	700 FPM	--	1000 FPM
B	AIR TEMPERATURE	80°C	--	100°C
C	TRAY LOADING	1.75 G/CM^2 (2.5 CM DEPTH)	--	3.50 G/CM^2 (5.0 CM DEPTH)
D	WATER/BEAN RATIO	0.8	1.0	1.2

were considered to be cooked when all three samples possessed a softness approximately equal to or greater than that of regularly cooked beans). The cooked beans were evaluated by a 15-member nonexpert panel, again utilizing a scale of 1–10, with respect to the following quality attributes: (1) flavor; (2) color; (3) appearance; (4) aroma; and (5) overall acceptance. As in the case of the cooking experiments, results of the drying experiments were analyzed by Analysis of Variance Techniques (Davies 1963), utilizing the triple and quadruple interactions as estimates of error. Separate analyses were performed for each attribute, including degree of butterflying.

Beans utilized in this research (*Phaseolus vulgaris*) were of the "pinto" variety, grown in the agricultural district of Cuauhtemoc, Chihuahua, Mexico and harvested in June, 1984. The entire lot of beans purchased for this research was homogenized by thorough mixing, after which beans were packed in burlap bags and stored in refrigeration (4–5 C) until used.

RESULTS AND DISCUSSION

Data obtained in the cooking experiments (Table 3) showed scores for the different quality attributes to vary from minimum values of 5–6 to maximum values of 8–9.

Table 4 reports results of Analysis of Variance calculations performed on the data obtained in the cooking experiments. Water/bean ratio was significant in all cases, with higher scores always corresponding to the high level. Cooking time was significant in the cases of color, flavor and overall acceptance, again with higher scores being assigned to the long cooking time. Cooking pressure was significant in the cases of color, aroma and overall acceptance; as in the former two cases, higher scores corresponded to the high pressure. Results are best summarized by noting that maximum organoleptic scores were obtained by the high levels of water/bean ratio, as well as of cooking time and pressure. The case of overall acceptance, in which all three variables exhibited a significant positive correlation with score, verifies this conclusion.

Data obtained in the drying experiments (Table 5) show that scores for the different quality attributes of the cooked beans varied from minimum values of 6.5–7.0 to maximum values of 8.0 to 8.5. Average scores of freshly cooked and dried recooked beans corresponding to the same quality attribute, i.e., color, flavor, aroma and overall acceptance, were compared by application of Analysis of Variance (F-test) techniques (Spiegel 1975). With all attributes, both sets of scores were found to be not significantly different from each other, indicating that panelists considered both samples to be equally acceptable. Score numerical values also indicated good acceptance of the dried recooked beans.

TABLE 3.
EXPERIMENTAL DESIGN MATRIX WITH CORRESPONDING RESULTS OBTAINED IN COOKING EXPERIMENTS

Experiment	A[a]	B[a]	C[a]	D[a]	Color[b]	Flavor[b]	Texture[b]	Aroma[b]	Overall Acceptance[b]
1	1.0	1 hr	25°C	10 psig	8.3±1.2	6.2±1.3	5.0±1.1	5.0±2.0	6.9±1.0
2	1.2	1	25	10	6.5±0.9	7.1±0.9	6.8±1.0	6.5±2.1	7.9±0.9
3	1.0	2	25	10	7.8±1.1	7.6±1.1	7.4±1.3	6.9±2.7	8.0±1.0
4	1.0	1	100	10	6.3±1.3	7.5±1.2	6.9±1.4	6.4±2.2	7.9±1.2
5	1.0	1	25	15	5.3±1.5	6.1±1.2	5.1±1.6	5.9±1.7	7.0±1.0
6	1.2	2	25	10	7.8±1.0	8.0±0.6	6.9±1.2	6.1±3.1	8.4±1.0
7	1.2	1	100	10	6.5±1.3	8.3±0.9	8.0±1.5	7.4±2.0	8.6±0.9
8	1.2	1	25	15	8.2±0.8	8.6±0.6	8.4±0.6	7.4±2.4	8.9±0.9
9	1.0	2	100	10	5.9±1.6	6.7±1.5	6.0±2.2	5.0±3.2	7.5±1.0
10	1.0	2	25	15	8.2±0.8	8.9±0.8	8.3±0.9	8.4±1.1	8.5±0.7
11	1.0	1	100	15	6.3±1.8	6.7±1.8	6.8±2.1	6.8±2.8	8.0±1.1
12	1.2	2	100	10	7.2±0.8	7.8±1.4	7.7±1.4	6.9±2.5	8.5±0.8
13	1.2	2	25	15	8.3±1.0	8.3±0.8	8.3±1.9	7.4±3.3	8.7±1.0
14	1.2	1	100	15	7.4±1.6	8.0±1.2	7.6±1.5	7.8±1.7	8.5±0.8
15	1.0	2	100	15	6.8±1.9	8.2±1.3	7.5±1.8	7.0±3.0	8.4±0.7
16	1.2	2	100	15	8.3±0.6	8.4±0.6	8.1±1.0	8.1±3.0	9.0±0.7

[a]Coding of main effects: A = water/bean ratio; B = cooking; C = soak water temperature; D = cooking pressure.
[b]Results reported as mean ± standard deviation.

TABLE 4.
RESULTS OF ANALYSIS OF VARIANCE CALCULATIONS PERFORMED ON DATA OF COOKING EXPERIMENTS

QUALITY	SIGNIFICANT FACTORS	SIGNIFICANCE LEVEL	MAGNITUDE OF EFFECT
FLAVOR	A: WATER/BEAN RATIO	$p < 0.05$	+0.8[a]
	B: COOKING TIME	$p < 0.05$	+0.7[a]
COLOR	A: WATER/BEAN RATIO	$p < 0.05$	+1.1[a]
	B: COOKING TIME	$p < 0.05$	+1.0[a]
	D: COOKING PRESSURE	$p < 0.10$	+0.7[a]
TEXTURE	A: WATER/BEAN RATIO	$p < 0.05$	+1.1[a]
AROMA	A: WATER/BEAN RATIO	$p < 0.10$	+0.8[a]
	D: COOKING PRESSURE	$p < 0.05$	+1.1[a]
OVERALL ACCEPTANCE	A: WATER/BEAN RATIO	$p < 0.01$	+0.8[a]
	B: COOKING TIME	$p < 0.05$	+0.4[a]
	D: COOKING PRESSURE	$p < 0.05$	+0.4

[a]Increment in average score

TABLE 5.
EXPERIMENTAL DESIGN MATRIX WITH CORRESPONDING RESULTS OBTAINED IN DRYING RUNS

Experiment	A[a]	B[a]	C[a]	D[a]	Color[b]	Flavor[b]	Appearance[b]	Aroma[b]	Overall Acceptance[b]	Butter-flying[c]
		°C								
1	700 fpm	80	2.5 cm	0.8	8.5±0.8	8.7±0.9	8.2±1.2	8.5±0.5	8.6±0.5	32%
2	1000	80	2.5	0.8	7.1±2.3	7.6±1.3	7.1±1.9	7.9±1.5	7.9±0.7	46
3	700	100	2.5	0.8	8.3±1.2	8.3±1.0	8.5±0.8	8.1±1.1	8.7±0.8	28
4	1000	100	2.5	0.8	7.3±1.0	6.8±2.3	6.7±1.8	7.9±1.4	7.7±1.5	29
5	700	80	5.0	0.8	7.2±1.5	7.1±1.4	6.9±1.8	7.6±1.3	7.5±1.1	24
6	1000	80	5.0	0.8	7.5±1.5	7.5±1.3	7.3±0.9	7.4±2.4	8.3±0.7	25
7	700	100	5.0	0.8	7.5±1.1	7.9±1.2	7.1±1.6	8.1±1.1	8.5±0.7	32
8	1000	100	5.0	0.8	8.1±0.9	7.8±1.0	7.7±1.2	7.8±1.1	8.2±0.7	16
9	700	80	2.5	1.0	5.9±1.8	6.2±1.9	6.1±2.1	7.5±1.4	7.5±1.3	30
10	1000	80	2.5	1.0	7.2±1.3	7.4±1.3	7.5±1.1	7.9±1.3	8.2±0.9	35
11	700	100	2.5	1.0	7.0±1.3	6.6±1.7	6.4±1.7	7.1±2.1	7.8±0.8	40
12	1000	100	2.5	1.0	7.3±0.9	7.0±1.1	6.9±1.5	7.9±1.3	8.0±0.5	24
13	700	80	5.0	1.0	7.8±0.8	7.7±1.3	7.5±1.5	7.8±0.9	8.1±0.6	45
14	1000	80	5.0	1.0	7.9±1.2	7.3±2.2	7.1±1.7	7.5±1.6	8.2±1.0	50
15	700	100	5.0	1.0	7.7±1.2	7.9±1.0	7.7±1.8	7.3±1.8	7.9±1.0	40
16	1000	100	5.0	1.0	7.7±1.4	6.9±1.9	7.2±1.9	7.4±1.8	8.1±0.8	40
17	700	80	2.5	1.2	7.9±1.3	8.0±0.9	7.3±2.6	7.8±1.1	8.5±0.7	60
18	1000	80	2.5	1.2	7.8±1.0	8.0±0.8	7.3±1.5	7.8±1.3	8.2±0.9	80
19	700	100	2.5	1.2	7.0±1.4	7.0±2.1	6.5±2.0	7.3±2.4	8.1±1.1	35
20	1000	100	2.5	1.2	7.3±1.7	6.9±1.6	6.0±2.4	6.0±3.0	7.7±0.9	90
21	700	80	5.0	1.2	7.9±0.8	7.5±0.9	7.2±1.3	8.1±0.8	7.8±0.9	65
22	1000	80	5.0	1.2	8.0±0.8	6.9±2.6	7.1±1.5	7.6±1.5	8.0±1.0	65
23	700	100	5.0	1.2	8.3±1.1	8.5±1.1	7.4±1.6	8.1±1.5	8.3±1.2	65
24	1000	100	5.0	1.2	7.8±1.6	7.3±1.9	6.8±1.9	7.4±1.7	7.9±1.0	65

[a]Coding of main effects: A = Air velocity, fpm; B = Air temperature (dry bulb), °C; C = Tray depth, cm; D = Water/bea ratio of beans before precooking.
[b]Results reported and mean ± standard deviation.
[c]Results reported as mean of two determinations.

Table 6 reports results of Analysis of Variance calculations performed on the data obtained in the drying experiments. Although a number of variables and interactions were found to be significant, with the exception of water/bean ratio in the case of degree of butterflying, magnitudes of corresponding effects were low, and in some cases negligible. Only tray depth and water/bean ratio were found to affect quality scores, with higher values corresponding to the high tray depth and low water/bean ratio. Thus, optimum dehydrated cooked bean quality should be obtained by utilizing a water/bean ratio of 0.8 and a tray depth of 5 cm, independently of air temperature and velocity. Degree of butterflying, on the other hand, was affected by all variables studied, although change of water/bean ratio from low to high level produced by far the greatest increment. Table 6 shows that minimum degree of butterflying should result from drying at 100 C, 700 fpm velocity and tray depth of 5 cm and, especially by utilizing the 0.8 water/bean ratio. Indeed, Fig. 1 shows that degree of butterflying was clearly a function of water/bean ratio (each value of degree of butterflying in the figure was obtained by averaging individual values corresponding to the same water/bean ratio in Table 5).

Finally, the cooking time of 4–5 min for dry beans was found to be relatively unaffected by drying conditions or water/bean ratio.

SUMMARY AND CONCLUSIONS

(1) Results obtained in this research indicate that it is possible to produce dehydrated quick-cooking beans which yield organoleptic scores comparable to those of freshly cooked beans.

(2) The process developed for obtaining quick-cooking beans of good quality consists of blanching raw, dry beans in boiling water until a water/bean ratio of 0.8 has been attained. The soaked beans are then cooked at 15 psig for 2 h, after which the cooked beans are dried with air at 100 C, and 700 fpm air velocity, utilizing a tray depth of 5 cm, until 10% has been attained. The most important process variable is water/bean ratio.

(3) The process described in (2) sharply decreases bean butterflying during drying, and yields a dry product which cooks in 4–5 min.

(4) Maximum organoleptic scores of cooked beans (color, flavor, texture and aroma) were obtained by the high levels of water/bean ratio, as well as of cooking time and pressure. Bean soak water temperature, after an initial 30-minute blanch in boiling water, had no effect on quality.

(5) Maximum organoleptic scores (color, flavor, appearance and aroma) of the recooked dehydrated product were obtained by the high level of tray depth and lowest level of water/bean ratio. Air drying conditions (temperature and velocity) had no effect on quality.

TABLE 6.
RESULTS OF ANALYSIS OF VARIANCE CALCULATIONS PERFORMED ON DATA OF DRYING RUNS

QUALITY ATTRIBUTE	SIGNIFICANT FACTORS	SIGNIFICANCE LEVEL	MAGNITUDE OF EFFECT
FLAVOR	D: WATER/BEAN RATIO	$p < 0.05$	-0.3^a
	INTERACTION AC	$p < 0.10$	-0.3^a
	INTERACTION BC	$p < 0.05$	$+0.5^a$
	INTERACTION CD	$p < 0.10$	$+0.1^a$
COLOR	C: TRAY LOADING	$p < 0.01$	$+0.5^a$
	D: WATER/BEAN RATIO	$p < 0.10$	$+0.1^a$
AROMA	D: WATER/BEAN RATIO	$p < 0.10$	-0.2^a
	INTERACTION AB	$p < 0.10$	$+0.1^a$
	INTERACTION CD	$p < 0.05$	-0.1^a
APPEARANCE	C: TRAY LOADING	$p < 0.05$	$+0.5^a$
	D: WATER/BEAN RATIO	$p < 0.05$	$+0.6^a$
	INTERACTION AC	$p < 0.05$	-0.4^a
	INTERACTION AD	$p < 0.10$	-0.3^a
OVERALL ACCEPTANCE	INTERACTION AC	$p < 0.05$	$+0.2^a$
	INTERACTION BC	$p < 0.10$	$+0.2^a$
DEGREE OF BUTTERFLYING	A: AIR VELOCITY	$p < 0.05$	$+4^b$
	B: AIR TEMPERATURE	$p < 0.10$	-4^b
	C: TRAY LOADING	$p < 0.10$	-2^b
	D: WATER/BEAN RATIO	$p < 0.01$	$+20^b$
	INTERACTION AC	$p < 0.01$	-3^b
	INTERACTION BC	$p < 0.10$	$+4^b$
	INTERACTION CD	$p < 0.05$	$+2^b$

[a]Increment in average quality score

[b]Increment in percent butterflying

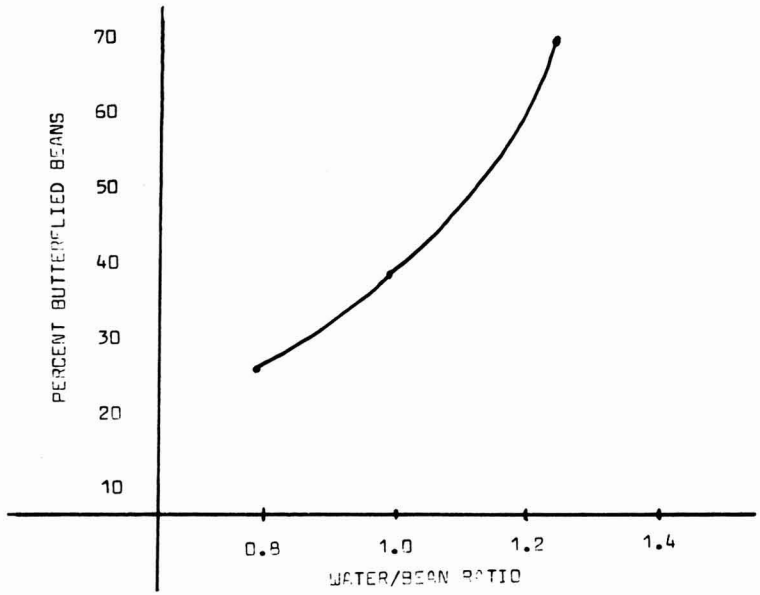

FIG. 1. PERCENT BUTTERFLIED BEANS AS A FUNCTION OF COOKED BEAN WATER/BEAN RATIO DURING DRYING

(6) Minimum bean butterflying, after cooking and drying, was obtained by the high levels of drying air temperature and tray depth, low level of drying air velocity, and lowest level of water/bean ratio. Butterflying was most affected by water/bean ratio.

(7) Cooking time of the precooked dried beans was apparently unaffected by the dehydration variables considered in this research. Average cooking times of the precooked dried beans were of the order of 4–5 min.

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EFFECT OF FROZEN STORAGE TEMPERATURE ON FREE AND BOUND FORMALDEHYDE CONTENT OF COD (*GADUS MORHUA*) FILLETS

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ABSTRACT

Samples from the same lot of commercially harvested and processed Northern Bank cod (Gadus morhua) *fillets were frozen stored at −30°C, −22°C, −15°C, −12°C, and a simulated industrial fluctuating temperature program for ca. 90 days. Perchloric acid extracts of the frozen premium grade fillets were used for the determination of free formaldehyde (HCHO) and dimethylamine (DMA). Both the formaldehyde dehydrogenase (EC1.2.1.46) enzyme and the Cochin and Axelrod (1959) modification of the colorimetric Nash (1953) method were used to compare the free HCHO content of the extracts. The enzyme procedure consistently detected 21% less HCHO in the fillets during the course of the study. Initial appearance of bound HCHO in the fillets, determined as the difference between DMA and enzymatically or colorimetrically assayed free HCHO, was influenced by frozen storage temperature. The lower concentration of HCHO detected by the enzyme method greatly influenced both the free HCHO concentration and the initial appearance of bound HCHO in the fillets. First-order reaction rates for free HCHO and DMA were higher at −12°C ($p \leqslant 0.05$) than those at −15°C, −22°C, or −30°C but lower than that of the fluctuating temperature conditions. Results also show that both the ratio of DMA and HCHO formation and the ratio of mean DMA and HCHO concentrations were > 1. A more accurate determination of free HCHO in frozen stored fish muscle could provide a better understanding of the role of bound HCHO in textural deterioration.*

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INTRODUCTION

As early as the 1940's, researchers began investigating the reaction of formaldehyde (HCHO) with proteins (Fraenkel-Conrat *et al.* 1945). Twenty years later, Amano and Yamada (1964; 1965) observed that the toughness of gadoid species increased with frozen storage temperature and time. The principal chemical implicated in the textural change was HCHO from the enzymatic breakdown of the osmoregulatory chemical trimethylamine oxide (TMAO), the reaction of which produces a 1:1 stoichiometric ratio of dimethylamine (DMA) and HCHO. These two metabolites have been used as indicators of gadoid frozen storage deterioration. DMA is the frozen storage index of choice since it can be extracted quantitatively (Ruiter and Wesemen, 1976; Tokunaga *et al.* 1977; Lundstrom and Racicot 1983) whereas HCHO can only be partially recovered from fish muscle tissue (Castell and Smith 1973; Poulter and Lawrie 1978; Radford and Dalsis 1982). Further clarification of formaldehyde's contribution to protein changes in fish muscle during frozen storage would clarify the toughening mechanism which may involve HCHO. In general, textural deterioration of fish muscle has led to decreased consumer acceptance of frozen fish and in particular, gadoid species.

The rate of DMA formation has been found to be species, muscle type (Castell 1971; Castell *et al* 1971; 1973), processing (Jensen 1979), packaging (Babbitt *et al.* 1972), and temperature dependent (Tokunaga 1964b; 1974; Dingle and Lall 1979a). These workers reported that the relative levels of DMA produced in various species was silver hake (*Merluccius bilinearis*) > cusk (*Brosme brosme*) > pollock (*Pollachius virens*) > cod (*Gadus morhua*) > haddock (*Melanogrammus aeglefinus*). Subsequently, Hultin (1981) showed that the DMA and HCHO production rate in red hake (*Urophycis chuss*) fillets stored at -18°C in polyethylene bags varied according to location, i.e., exterior vs interior and tail vs head.

Since the work of Fraenkel-Conrat *et al.* (1945), HCHO has been implicated as a causative agent in protein crosslinking in frozen stored fish (Fraenkel-Conrat, 1948; Fraenkel-Conrat and Olcott 1948; Mihalyi 1963; Connell 1975; Galembeck *et al.* 1977; Kelly *et al.* 1977). Current literature suggests that HCHO binds preferentially to low molecular weight compounds in fish (Banda and Hultin 1983). If HCHO binds with such compounds, then the implications of HCHO bonding in the frozen storage textural toughening of fish flesh (Gill *et al.* 1979) are still inconclusive.

Current analytical procedures for the determination of HCHO in fish muscle are based on the modification (Cochin and Axelrod 1959) of the colorimetric Nash (1953) reagent method. In this method, neutralized trichloroacetic acid (TCA) or perchloric acid ($HClO_4$) extracts of fish muscle are assayed by deproteination and extraction of free HCHO prior to color formation. An advantageous alter-

native procedure (LeBlanc *et al.* 1988) would be to use an enzyme that is specific for HCHO. Commercial availability of formaldehyde dehydrogenase (EC 1.2.1.46) prompted us to further evaluate this enzyme in comparison with the Cochin and Axelrod (1959) colorimetric HCHO method to determine bound HCHO on samples from the same lot of harvested and processed Northern Bank trawl cod (*Gadus morhua*) stored at simulated industrial fluctuating conditions and comparable constant frozen storage temperatures for ca. 90 days.

METHODS

Experimental Design

Fish samples were obtained from the same lot of commercially harvested and processed Northern Bank cod (*Gadus morhua*) produced by Fishery Products Ltd. The premium grade individually wrapped fillets were packaged in 2.2 kg polyethylene paperboard cartons overwrapped in corrugated paperboard cases. The fillets were taken from bled, gutted Northern Bank trawl cod which had been held in ice for 48h after death to permit the resolution of rigor. At this time the fish were filleted, packaged as stated, and frozen in a plate freezer at −40°C for 3-4 h with subsequent storage at −30°C prior to air shipment on dry ice from Newfoundland to Halifax, Nova Scotia. Same day shipments were transported immediately from the airport to the laboratory. The fillets were randomly distributed among microprocessor computer controlled temperature compartments at −30°C, −22°C, −15°C, −12°C and a fluctuating temperature storage unit with a program as follows: −30°C (14 days), −15°C (4 days), −22°C (21 days), −12°C (21 days), and −30°C for the remaining storage period. Moisture determinations and preparation of $HClO_4$ extracts were done as described under *Chemical Analyses*. The $HClO_4$ extracts were analyzed to compare the Cochin and Axelrod (1959) modification of the Nash (1953) procedure and the HCHO dehydrogenase (EC 1.2.1.46) enzyme method on samples expected to have increased HCHO levels due to the frozen storage conditions. Dimethylamine-nitrogen (DMA-N) (Castell *et al.* 1974) content was determined in duplicate on the same $HClO_4$ extracts for each sampling period to compare results with the HCHO methodology assessed.

Chemical Analyses

Moisture. Moisture content was determined in triplicate on ca. 15 g fish samples placed in aluminum foil dishes (Horowitz 1980). The samples were dried to constant weight at 110°C in a forced air convection oven, cooled in a desiccator and weighed. The loss in weight was reported as percent moisture content. This value was used in the calculation of the HCHO and DMA content of the samples.

$HClO_4$ Extracts of Frozen Fish Fillets. The frozen fillets were thawed overnight in a refrigerator at 4 °C and were sampled as follows. The thawed fish fillets were cut into 1 cm^3 pieces, mixed, and 60 g samples were homogenized in 120 mL of 6% (w/v) $HClO_4$ (A.C.S. reagent grade, Fisher Scientific Company) (Ehira *et al.* 1970). Duplicate pairs of extracts were prepared from replicate halves of the same 454 g package of fillets. Homogenization was accomplished by blending at maximum speed in a precooled Cuisinart (Model RC1) for two, one minute periods and the walls of the food processor jar were scraped down at the end of each period. The homogenates were filtered immediately through Whatman No. 1 filter paper and a 100 mL aliquot of the filtrate was adjusted to pH 7.0 with a measured volume of 30% (w/v) KOH (A.C.S. reagent grade, Fisher Scientific Company). The neutralized extract was refrigerated for 1h to allow $KClO_4$ crystals to precipitate. Suitable aliquots of the extract were placed into vials, frozen, and stored at −30 °C for subsequent analysis at which time they were thawed overnight in a refrigerator at 4 °C.

Formaldehyde. Assays for free HCHO using the Cochin and Axelrod (1959) modification of the Nash (1953) reagent method were performed in duplicate on each of the $HClO_4$ extracts described. Absorption of the standards and samples was read at 400 nm using a double beam Pye-Unicam SP 800 spectrophotometer.

Assays for free HCHO using the HCHO dehydrogenase enzyme method developed by Yasuhara *et al.* (1982) for creatine determination were performed in duplicate on each of the $HClO_4$ extracts described. Thirty mg HCHO dehydrogenase (EC 1.2.1.46) (Cat. No. F-1879, Sigma Chemical Co.) and 20 mg β-nicotinamide adenine dinucleotide (NAD) (Cat. No. N-7004, Sigma Chemical Co.) were combined with 200 mL 0.1 M phosphate buffer, pH 7.8. For each sample, the absorbance of a 3 mL aliquot of HCHO dehydrogenase-NAD phosphate buffer solution (ca. 22 °C) was measured at 340 nm using the spectrophotometer described previously. A 0.5 mL sample of the neutralized $HClO_4$ extract was added, quickly mixed using a disposable Pasteur pipette, and after 30 min the absorbance was read at 340 nm.

Bound HCHO was calculated for both the modified Nash and HCHO dehydrogenase (EC 1.2.1.46) enzyme methods as the difference between values for DMA and free HCHO, based on the assumption that DMA and HCHO are produced stoichiometrically from TMAO in a 1:1 ratio.

Dimethylamine. Assays for DMA using the method of Castell *et al.* (1974) were performed in duplicate on the same $HClO_4$ extracts for each sampling period. The DMA content of the fish muscle was calculated using the standard formula and a calibration curve prepared with known amounts of DMA.

Statistical Analyses. The data obtained from the experiments were subjected to appropriate statistical analyses using the Statistical Analysis System (SAS Institute, Inc. 1987) package and plotted using the Zeta-8 plotter on the Vax 8200

mainframe computer system at Mount Saint Vincent University. First-order rates were calculated by logarithmic transformation.

RESULTS AND DISCUSSION

Premium grade cod (*Gadus morhua*) fillets stored under a set of industrial fluctuating temperature conditions and at similar controlled constant temperatures for ca. three months were analyzed for free HCHO and DMA content as chemical indicators of frozen fish quality. Figures 1 and 2 show the results of the free HCHO and DMA analysis on the fillets subjected to the described conditions.

There was a steep initial rise in free HCHO concentration during the first three weeks of storage followed by a levelling effect for the −12°C, −15°C, and

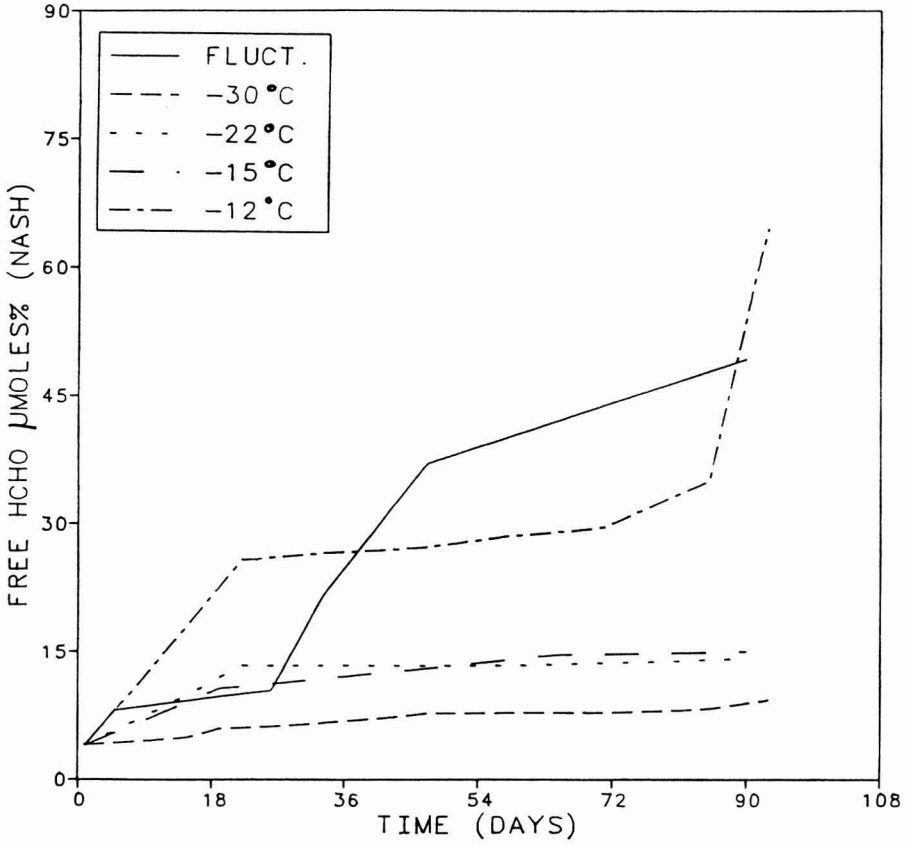

FIG. 1. MODIFIED NASH DETERMINATION OF FREE HCHO CONTENT OF THE COD (*GADUS MORHUA*) FILLETS SUBJECTED TO THE VARIOUS STORAGE CONDITIONS

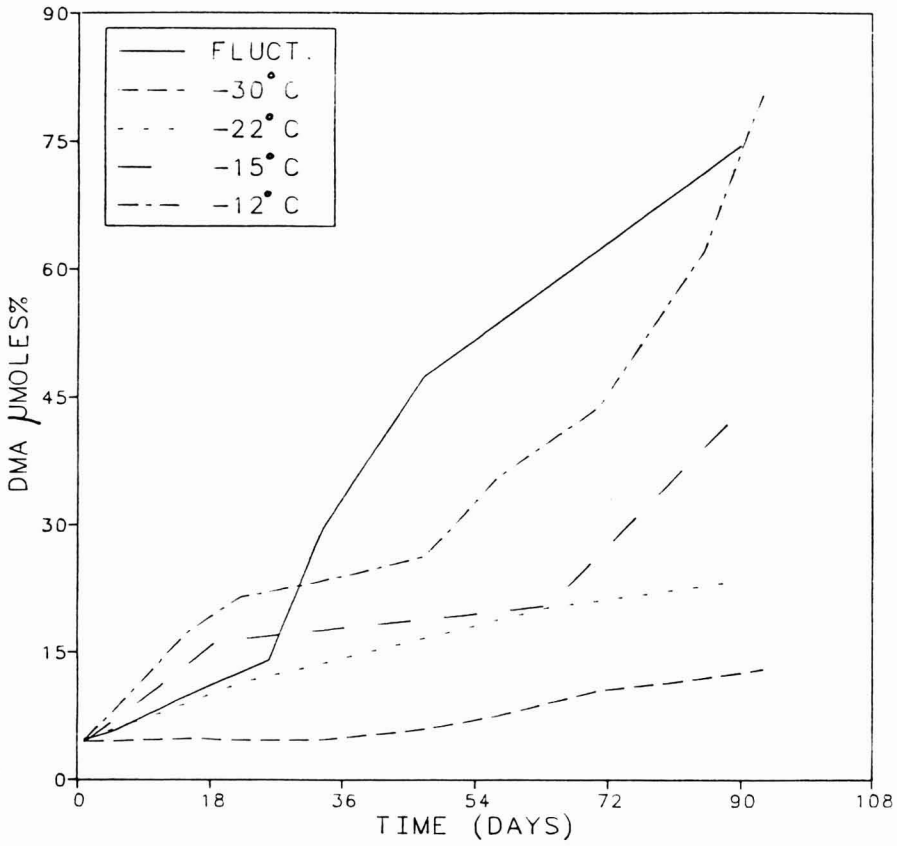

FIG. 2. DIMETHYLAMINE CONTENT OF THE COD (*GADUS MORHUA*) FILLETS SUBJECTED TO THE VARIOUS STORAGE CONDITIONS

−22 °C treatments. Fillets subjected to the fluctuating temperature conditions showed a delayed steep rise in free HCHO concentration and those held at −12 °C showed a dramatic increase in HCHO content on the 93rd day of the storage study. The present profile of free HCHO formation in cod fillets is similar to the DMA profile of cusk (*Brosme brosme*) reported by Dingle and Lall (1979b); Figure 2 shows that DMA increased with storage time for all treatments but changes were negligible at −30 °C. This finding reflects those of various researchers (Banks 1965; Castell *et al.* 1970; 1975; Dingle *et al.* 1977) who have reported that temperatures between −26 °C and −30 °C are required to control deterioration in most fish species.

Results of the enzymatic analysis of free HCHO by the formaldehyde dehydrogenase method were highly correlated with the concentrations of free HCHO determined by the modified Nash colorimetric method (Fig. 3). The linear regression equation of the HCHO (Nash) to HCHO (Enz.) was

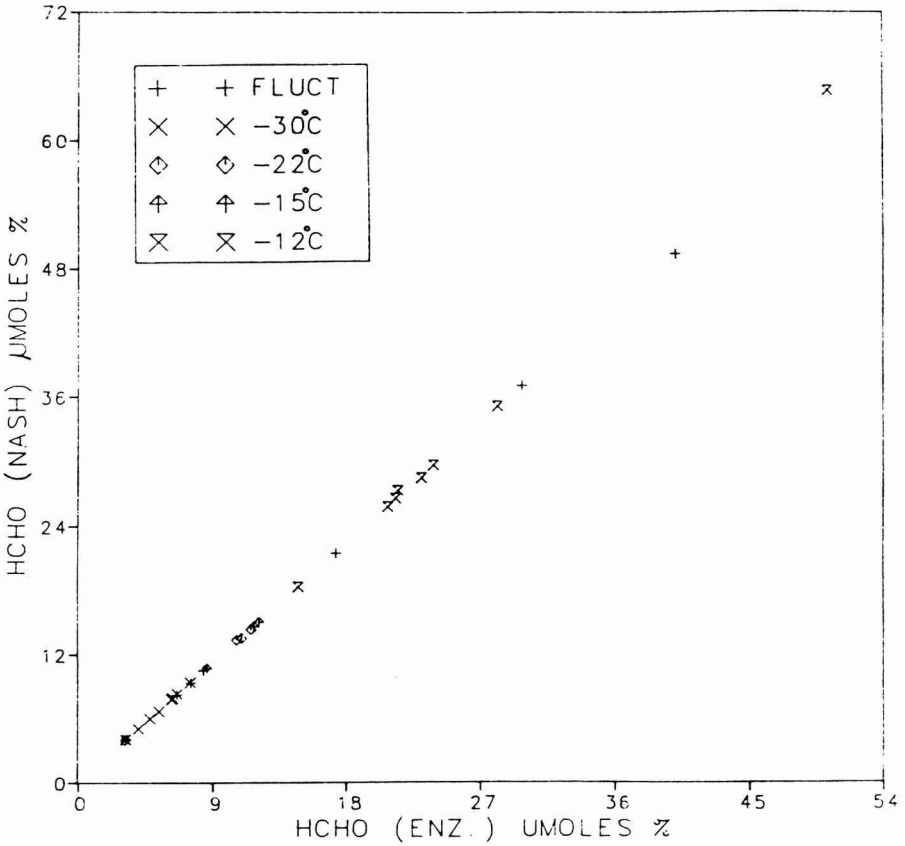

FIG. 3. CORRELATION OF HCHO DETERMINED BY THE MODIFIED NASH AND HCHO DEHYDROGENASE METHODS IN COD (*GADUS MORHUA*) FILLETS SUBJECTED TO THE VARIOUS STORAGE CONDITIONS

$$\text{HCHO (Nash)} = -0.123 + 1.26\ \text{HCHO (Enz.)}$$
$$\text{where } r = 1.0,\ n = 33;$$

HCHO (Enz.) was found to be present in the fillets at 79% of the modified Nash determined HCHO concentrations for all samples from this same lot of fish stored under the present test conditions. The slope value of 1.26 is similar to the slope value of 1.11 obtained with the standard addition HCHO procedure that was used to analyze various cod samples of convenience (LeBlanc *et al.* 1988). The difference between the two slope values may be due to initial fish quality, handling, icing, and any other treatment imposed on the fillets from production to sample analysis. The ratio of HCHO (Nash) : HCHO (Enz.) concentrations may indicate the quality of the fish prior to frozen storage.

Differences between the colorimetric Hantzsch reaction and the HCHO dehydrogenase method may be due to reagent selectivity. The colorimetric reaction does not distinguish between HCHO and acetaldehyde (Nash 1953). The two colored compounds produced have similar absorptivities near the same wavelength maxima whereas HCHO dehydrogenase has limited specificity toward acetaldehyde and glyoxal (Ando *et al.* 1979). The accuracy of the colorimetric method for HCHO analysis of frozen fish muscle may be limited by the presence of acetaldehyde (McGill *et al.* 1977; Ikeda 1980), a metabolic precursor of ethanol formation (Ahamed and Matches 1983; Kelleher and Zall 1983). Nevertheless, the level of HCHO may be more accurately determined by the enzymatic procedure because the results do not depend on the formation of a colored complex. More research on the enzymatic determination of free HCHO must be carried out to identify potential interferences and overall procedural accuracy. The major implication of these methodology findings is in the determination of HCHO bound to fish muscle protein or to NPN compounds and their relationship to the toughening process in gadoid cod muscle during frozen storage.

Bound HCHO was determined as the difference between DMA content (equivalent to the total HCHO produced in the fillets) and the free HCHO determined in the same extracts. Figures 4 and 5 show the changes in bound HCHO with frozen storage temperature and time determined by the respective colorimetric and enzymatic methods. Regardless of the method, the initial appearance of bound HCHO is storage temperature dependent (Fig. 4). Because the HCHO determined enzymatically is lower than the HCHO determined by the modified Nash method, bound HCHO increases proportionally. Unlike the results presented in Fig. 4, bound HCHO determined enzymatically (Fig. 5) increases with storage time over the entire study. Bound HCHO was not found in fillets stored at $-30\,^{\circ}C$ from days 18 to 45 regardless of the method of analysis. The highest increase in bound HCHO appears in fillets stored under the fluctuating temperature treatment. The higher bound HCHO values determined enzymatically suggest that more HCHO may be covalently bound to proteins or with NPN compounds (Banda and Hultin 1983) or both. This could partly explain increases in fish muscle toughness during frozen storage. The higher bound HCHO values which result from the lower free HCHO concentrations determined enzymatically may shed light on the changes occurring in the early stages of fish muscle toughening.

Kelleher *et al.* (1982) suggested that bound HCHO was an important chemical index but sample variation limits its usefulness as a quality indicator for gadoid species. Nevertheless, their results on blocks of red hake (*Urophycis chuss*) fillets stored at $-18\,^{\circ}C$ for 24 weeks showed a steady increase from ca. 0 mmoles% to ca. 2 mmoles% bound HCHO. It is clear from literature findings that species and temperature greatly influence the rate and level of bound HCHO content found in fillets or minced tissue. In data extrapolated from Tokunga (1964a) no bound HCHO was found in Alaska pollock stored at $-17\,^{\circ}C$ to $-19\,^{\circ}C$ for 75 days. Cod stored for ca. 190 days at $-15\,^{\circ}C$ exhibited bound HCHO levels of 0.33

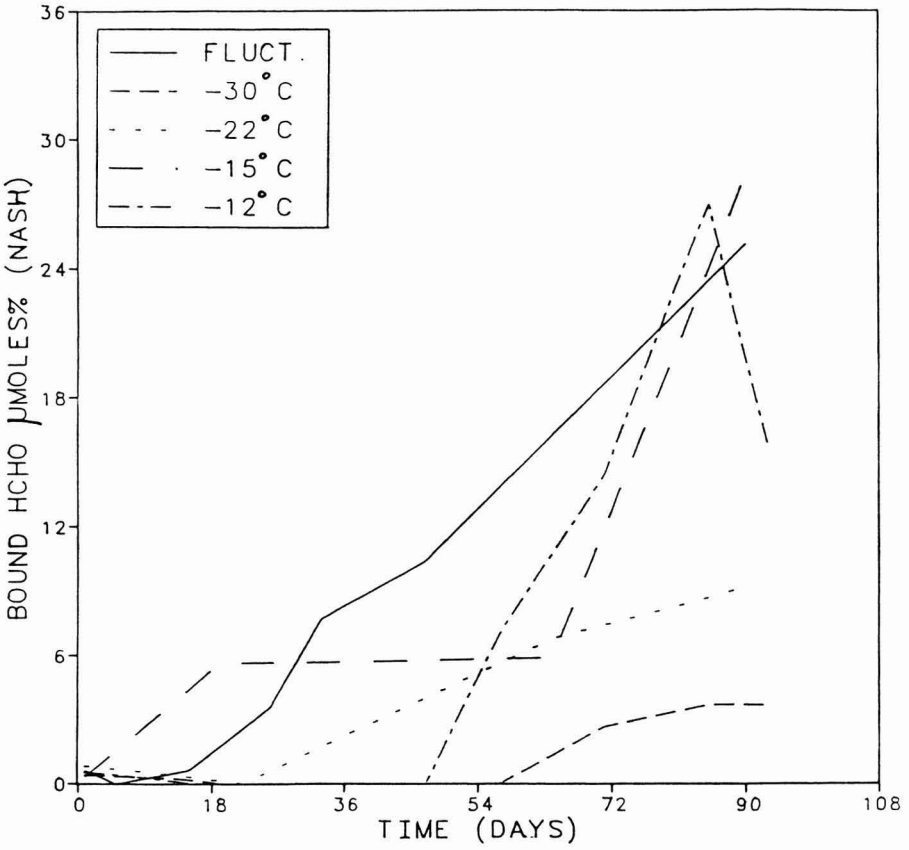

FIG. 4. COLORIMETRIC DETERMINATION OF BOUND HCHO CONTENT OF THE COD (*GADUS MORHUA*) FILLETS SUBJECTED TO THE VARIOUS STORAGE CONDITIONS

mmoles% (Mackie and Thomson 1974). Conversely, work by Crawford *et al.* (1979) showed that during 12 months of storage, bound HCHO in true cod (*Gadus macrocephalus*) remained fairly constant whereas in Pacific hake (*Merluccius productus*) it increased exponentially from 6 to 202 μmoles%. In addition, a temperature dependent relationship for bound HCHO is further supported by the data calculated from Gill *et al.* (1979). Their results on red hake showed that bound HCHO increased faster in fillets stored at −5°C compared to those stored at −17°C for 40 days. Parkin and Hultin's data (1982) showed that bound HCHO increased twice as fast in minced red hake fillets compared to intact fillets with a plateau occurring between day 7 and day 56. Further work (Owusu-Ansah and Hultin 1986) showed that bound HCHO in red hake remained constant during the first 80 days of storage after which its formation accelerated exponentially.

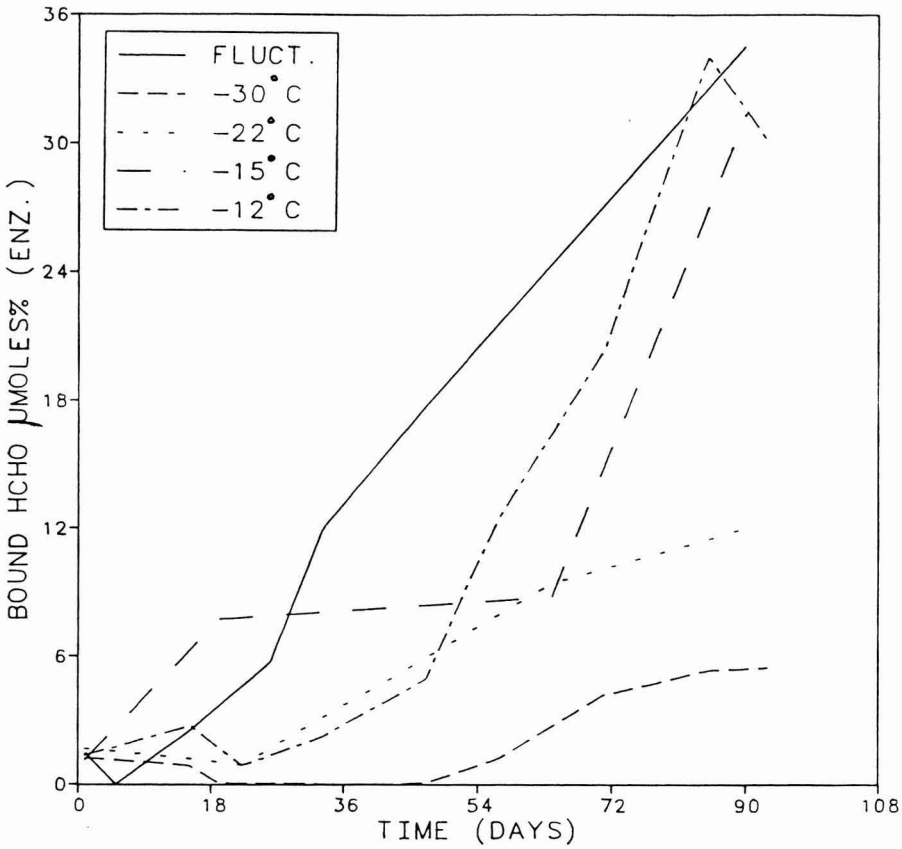

FIG. 5. ENZYMATIC DETERMINATION OF BOUND HCHO CONTENT OF THE COD (*GADUS MORHUA*) FILLETS SUBJECTED TO THE VARIOUS STORAGE CONDITIONS

Data calculated at the various temperatures for the same lot of fish subjected to the storage conditions in the present study offer a clearer perspective of the pattern of bound HCHO changes inherently occurring in frozen gadoid muscle (Fig. 4 and 5). But a problem still exists for the accurate determination of bound HCHO. Castell and Smith (1973) reported respective mean HCHO recovery values of 53 and 49% for homogenized cod tissue extracted with either TCA or $HClO_4$. Employing similar procedures on whiting (*Merlangius merlangus*) stored at −8°C, Poulter and Lawrie (1978) found a 60% extraction efficiency which increased with storage time whereas Radford and Dalsis (1982) extracted 72% in fresh iced shrimp (*Penaeus setiferus*). Unfortunately, such recovery procedures do not reflect the actual fish muscle matrix extraction efficiency. The decrease in HCHO recovery may be due to either reaction with proteins or NPN components or both. Unpublished results from our laboratory on fresh fish fillets dipped in room

temperature solutions of HCHO showed increased Instron Kramer shear values and decreased sensory textural quality after only 18 h of storage at 4 °C. Conversely, extraction of the stable DMA metabolite has been found to be nearly quantitative (Ruiter and Wesemen 1976; Tokunaga *et al.* 1977; Lundstrom and Racicot 1983). Furthermore, bound HCHO would become nonexistent or minute in quantity when free HCHO values are corrected for a reported extraction efficiency of 49-72% (Castell and Smith 1973; Poulter and Lawrie 1978; Radford and Dalsis 1982). Present results based on these recovery factors show that no HCHO is covalently bound at either −15 °C or −12 °C until after ca. 80 days storage. Therefore, this observation would imply that during this length of frozen storage HCHO is not a major causative factor in toughening.

Equimolar concentrations of free HCHO and DMA are produced from the enzymatic breakdown of the osmoregulatory compound TMAO. Table 1 shows that the rates of free HCHO and DMA formation in cod fillets stored at −12 °C were higher ($p \leqslant 0.05$) than those of the other constant temperature storage treatments. DMA production in the fillets stored at −30 °C was lower ($p \leqslant 0.05$) than that of the other storage conditions. The respective free HCHO and DMA rates for the fillets stored at −12 °C were 2.4 and 1.8 times higher than that for the fillets stored at −30 °C whereas the fluctuating storage treatment was 3.3 and 2.5 times greater. Raising the temperature even for short periods of time, regardless of subsequent reduction, promotes diffusion and redistribution of reactants and metabolites, facilitating the occurrence of deteriorative reactions. Temperature fluctuations also promote ice recrystallization and increased crystal size that results in additional damage to membranes separating cell constituents. This may liberate enzymes and metabolites from the mitochondria and other cell inclusions, many

TABLE 1.
ANALYSIS OF COVARIANCE OF FIRST-ORDER REACTION RATES FOR HCHO AND DMA PRODUCTION, RATIO OF THESE RATES, AND RATIO OF MEAN CONCENTRATIONS IN COD (*GADUS MORHUA*) FILLETS SUBJECTED TO THE VARIOUS STORAGE CONDITIONS

	TREATMENT[1]				
ANALYSIS	Fluctuating	-30°C	-22°C	-15°C	-12°C
HCHO day^{-1}	0.026a	0.0078c	0.012bc	0.013bc	0.019b
DMA day^{-1}	0.032a	0.013c	0.017b	0.021b	0.024b
DMA/HCHO rates	1.2	1.7	1.4	1.6	1.3
[DMA]/[HCHO]	1.4	1.1	1.9	1.4	1.2

[1]Values in the same row with the same letter are not significantly different ($p \leqslant 0.05$).

of which are maximally active in the tissue held at elevated frozen storage temperatures (Matsumoto 1980; Fennema 1982).

Reaction rates for the cod fillets from the same lot of fish subjected to the various storage conditions of the present study (Table 1) are quite different from those calculated using published literature (Table 2). True cod (*Gadus macrocephalus*) fillets wrapped in moisture vapor-proof film and stored at −26 °C produced free HCHO and DMA, respectively, at a rate of 0.0016 and 0.0010 day^{-1} (Crawford *et al.* 1979). Using nearly identical storage temperatures, Parkin and Hultin (1982) showed that the rates of free HCHO and DMA production in red hake were higher than those found for the same species by Owusu-Ansah and Hultin (1986). Although not presented in Table 2, data of Castell *et al.* (1971) showed that a

TABLE 2.
COMPARISON OF CALCULATED FIRST-ORDER REACTION RATE LITERATURE VALUES FOR DIFFERENT SPECIES OF FROZEN FILLETS SUBJECTED TO VARIOUS STORAGE TEMPERATURES

ANALYSIS	Cod		True cod	Red hake		Silver hake
	-15°C[1]	-5°C[2]	-26°C[3]	-7°C[4]	-6°C[5]	-15°C[6]
HCHO day-1	0.0050	-	0.0016	0.0066	0.026	-
DMA day-1	0.0072	0.034	0.0010	0.016	0.033	0.0055

[1]Mackie and Thomson 1974.
[2]Castell *et al.* 1971.
[3]Crawford *et al.* 1979.
[4]Owusu-Ansah and Hultin 1986.
[5]Parkin and Hultin 1982.
[6]Licciardello *et al.* 1980.

mixture of dark and white muscle of cod (*Gadus morhua*) stored at −5 °C had a lower first-order DMA production rate than that for separated white muscle (0.063 vs 0.084 day^{-1}; similarly, white muscle fillets from red hake produced DMA at a slightly higher rate than whole fillets (0.039 vs 0.036 day^{-1}. In addition, the rate of DMA production in minced silver hake (*Merluccius bilinearis*) muscle (Licciardello *et al.* 1980) was higher than that for whole fillets. These researchers postulated that heme pigments may catalyze the formation of DMA and HCHO in dark muscle but Dyer and Hiltz (1974) did not find that removal of dark muscle affected the rate of deterioration in red hake. Thus, results calculated from the literature as well as those from the present study show that the rates of formation of free HCHO and DMA are species, muscle type, vapor barrier, temperature, storage time, preparation method (Castell *et al.* 1971; Mackie and Thomson 1974; Crawford *et al.* 1979; Licciardello *et al.* 1980; Parkin and Hultin 1982b; Owusu-Ansah and Hultin 1986), and for free HCHO, method of

analysis dependent (LeBlanc *et al.* 1988). Furthermore, HCHO rates (Tables 1 and 2) do not correspond to the DMA production.

To gain a better understanding of this discrepancy, the ratio of the first-order rates of DMA and HCHO formation and the ratio of the means for the tissue concentrations of DMA and HCHO during storage (Table 1) were compared with calculated literature findings (Table 3). The data show that the ratio of DMA:HCHO production is species, processing, temperature, and muscle type dependent (Tokunaga 1964b; Mackie and Thompson 1974; Tokunaga 1974; Gill *et al.* 1979; Owusu-Ansah and Hultin 1986). Present findings as well as the generally high ratio of DMA/HCHO calculated from published literature may be explained by the disappearance of HCHO that is chemically bound to proteins (Fraenkel-Conrat *et al.* 1945; Castell *et al.* 1973; Brunn and Klostermeyer 1983) or by reaction with low molecular weight nonprotein nitrogen compounds (NPN) (Banda and Hultin 1983). Conversely, Dingle and Lall (1979a) found no increase in the DMA/HCHO ratio for minced cusk (*Brosme brosme*); in fact, HCHO was 20% less than DMA in all the samples analyzed. No change in the ratio was believed indicative of the muscle proteins still possessing reactive groups that could combine with HCHO. This is supported by the present results which found that the ratio of the [DMA]/[HCHO] varied with storage temperature and time (Fig. 6).

This study has shown that the colorimetric procedure typically used for determination of HCHO in fish muscle extracts overestimates free HCHO and underestimates bound HCHO. But the former methodology does correlate well with results on free HCHO determined by the formaldehyde dehydrogenase enzyme procedure. If a recovery correction factor is used in the analysis and calculation of free HCHO content, it becomes evident that HCHO does not become bound in frozen fish muscle until ca. $\geqslant$ 80 days storage even though it has been implicated as a major cause of gadoid toughness. In addition, the fluctuating temperature conditions exhibited the greatest rate ($p \leqslant 0.05$) of deterioration when compared to constant temperature storage treatments.

ACKNOWLEDGMENTS

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TABLE 3.
COMPARISON OF CALCULATED LITERATURE VALUES FOR DMA AND HCHO FIRST-ORDER REACTION RATE RATIOS AND CONCENTRATIONS

ANALYSIS	Cod fillets[1]	Red hake fillets[2]	Minced red hake[2]	Red hake fillets[3]		Red hake fillets[4]	Red hake fillets[5]	Pacific hake fillets[6]	Alaska pollock fillets[7]
	-15°C	-6°C	-6°C	-5°C	-17°C	-7°C	-18°C	-26°C	-18°C
DMA/HCHO rates	1.4	1.3	1.2	0.70	0.59	2.4	1.0	0.63	1.2
[DMA]/[HCHO]	6.4	2.6	2.8	3.7	3.8	4.0	3.0	1.6	1.2

ANALYSIS	Minced dark muscle, Alaska pollock[8]			Minced light muscle, Alaska pollock[8]			Minced Grenadier[9]
	-5°C	-10°C	-20°C	-5°C	-10°C	-20°C	-10°C
DMA/HCHO rates	0.98	1.0	0.68	2.6	1.2	1.9	0.82
[DMA]/[HCHO]	2.6	2.0	2.2	4.3	2.5	2.8	5.3

[1]Mackie and Thomson 1974.
[2]Parkin and Hultin 1982.
[3]Gill *et al.* 1979.
[4]Owusu-Ansah and Hultin 1986.
[5]Kelleher *et al.* 1982.
[6]Crawford *et al.* 1979.
[7]Tokunaga 1964a.
[8]Tokunaga 1974.
[9]Dingle and Lall 1979

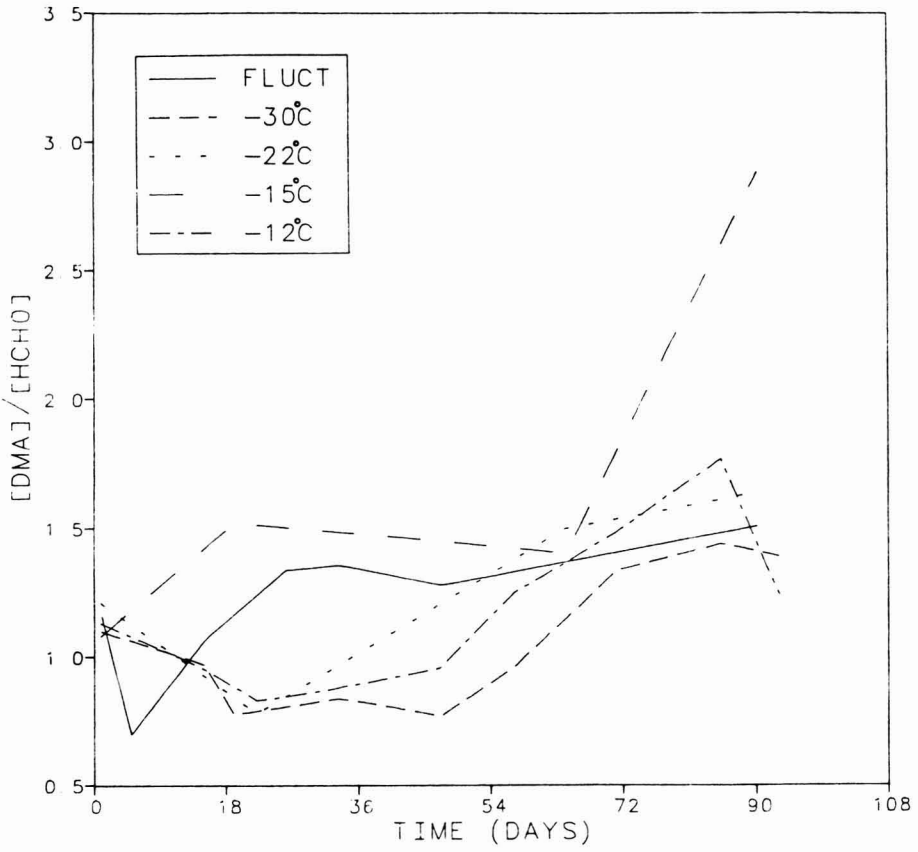

FIG. 6. RATIO OF DMA TO HCHO CONTENT OF COD (*GADUS MORHUA*) FILLETS SUBJECTED TO THE VARIOUS STORAGE CONDITIONS

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EFFECTS OF PROCESSING AND STORAGE ON THE PANTOTHENIC ACID CONTENT OF SPINACH AND BROCCOLI

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ABSTRACT

Free and total pantothenic acid (FPA and TPA) in spinach and broccoli were determined at various processing stages. Steam-blanching resulted in greater TPA retention thatn water-blanching. Spinach retained 36% TPA activity after water-blanching compared with 87% retention after steam-blanching. Similar trends were noted for TPA loss during blanching of broccoli. Canning of water-blanched spinach resulter in a further significant loss ($\alpha = 0.05$) of TPA. TPA was stable in canned spinach and frozen spinach. Frozen storage of broccoli for 60 days at $-32.2°C$ resulted in loss of TPA. No further loss was observed between 60 and 160 days of frozen storage. FPA was lost to a greater extent than TPA during water-blanching of both vegetables.

INTRODUCTION

Some review articles (Ratnatunga *et al.* 1978; Harris and Karmas 1975; Nesheim 1974; Barratt 1973; Cain 1967; Chichester 1971) are available regarding processing effects on the nutritional values of foods. However, there is a notable lack of information on the pantothenic acid contents of cooked and processed foods. Some information on pantothenic acid can be found in USDA Agricultire Handbook No. 97 (Zook *et al.* 1956), Home Ecomonics Research Report Number 36 (Orr 1969), and the revised Agriculture Handbook No. 8 (1976-1984). Most of the pantothenic acid data in these sources are on raw foods. More recent data on the pantothenic acid content of processed foods were reported by Walsh *et al.* (1981). They examined 75 foods on the ready-to-eat state for pantothenic acid content. These authors found a relatively low amount of pantothenic acid in many

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highly processed foods. Schroeder (1971) reported losses of pantothenic acid in frozen and canned foods, the results being based on the values taken from USDA Home Economics Research Report No. 36 (Orr 1969), and not from controlled studies (Orr and Watt 1972).

Comparisons have been made between steam and hot water blanching of vegetables with respect to nutrient and quality losses (Guerrant *et al.* 1947; Wedler 1971; Raab *et al.* 1973; Hein and Hutchings 1971; Freeman and Sistrunk 1973; Sistrunk and Bradley 1975; De Souza and Eitenmiller 1986). Higher retentions of nutrients and better quality have been consistently observed with steam blanching. As far pantothenic acid is concerned, there is little information about the blanching effect. Morgan *et al.* (1944) reported no loss in pantothenic acid in blanched commercially produced vegetables. It should be noted that the double enzyme system of phosphatase and pigeon liver extract had not yet been developed and used for the analysis of pantothenic acid. Therefore, the results are questionable. Morgan *et al.* (1945) performed steam blanching and water blanching of broccoli for 5 min. Although no exact numbers were given, the pantothenic acid retention can be roughly estimated using the graph presented to be 75% for steam blanching and 53% for water blanching. In the experiment of Bankhead *et al.* (1978), more than 50% of pantothenic acid in dehulled soybeans was lost in blanching with tap water or with 0.25% sodium bicarbonate solution. The latter treatment caused more loss of pantothenic acid than the former.

Steam cooking of blackeyed peas was reported by Kilgore and Sistrunk (1981) to retain more pantothenic acid and other B-vitamins than cooking by boiling if the peas had been soaked in phosphate or bicarbonate solution. No signifigant differences in pantothenic acid retention was observed between the steam cooked and boiled peas soaked in citrate buffer. Pearson and Lueke (1945) reported that 77% of the pantothenic acid was retained in sweet potatoes when cooked by baking and 100% retention when sweet potatoes were boiled. Dunn and Goddard (1948) studied the effect of heat upon pantothenic acid content of peanuts. They found that boiling peanuts for 40 min resulted in a loss of approximately 25%, and roasting at 180°C for the same period of time destroyed almost all the pantothenic acid while roasting at 160° resulted in a 25% loss.

Canning also results in loss of pantothenic acid from foods. Canned, combination fish and vegetable products (Wituszynska 1973) contained 60% less pantothenic acid than the fresh material from which they were made. Industrially canned Dutch Army meals (consisting of meat, vegetables, pulses and potatoes) were examined by Hellendoorn *et al.* (1971) for their nutritive values. Approximately 19% of pantothenic acid was lost after canning. Schroeder (1971) calculated mean percentage loss of certain nutrients in processed foods from the data compiled by Orr (1969). According to Schroeder's report, in canned foods of animal origin, losses of pantothenic acid ranged from 20% (seafood) to 35% (dairy products), and in canned vegetables, the loss of pantothenic acid was about

56%. These losses represent the sum of the losses during the entire canning process. The losses due to blanching can be quite large (Lund 1971).

Storage of canned foods leads to changes in the nutrient content. The extent of the changes is dependent on the temperature and time of storage and the product characteristics. More than 50% pantothenic acid was lost during 1 year of refrigerated storage of canned products made from fish and some vegetables (Wituszynska 1973). When stored at room temperature (22° ± 2°C), canned Dutch Army meals (Hellendoorn *et al.* 1971) lost approximately 50% of their pantothenic acid in 5 years of storage.

Pantothenic acid is a water soluble and heat labile vitamin. Commercial processing can inevitably cause losses of pantothenic acid from foods by leaching and/or destruction. However, the reported data are quite limited, sometimes conflicting and inconclusive. This is largely due to the difficulties and differences in the assay methods. A considerable amount of data available were obtained before the development of the now commonly used double enzyme system of phosphatase and pigeon liver extract treatment prior to microbiological assay to liberate pantothenic acid from the major bound form, coenzyme A. Therefore, the true picture of processing effects on the pantothenic acid content in foods needs more information to be delineated.

The objective of this study was to define processing effects on pantothenic acid content of two commonly consumed vegetables, spinach and broccoli. Specific objectives were to compare two common blanching treatments (water and steam) on the retention of pantothenic acid and to determine the effects of canning (spinach only), freezing and storage on pantothenic acid content.

MATERIALS AND METHODS

Sources of Spinach and Broccoli

Fresh California spinach purchased from a local wholesale product distributor was stored at mean temperature of 1.1 °C until prepared for processing. Samples were usually processed within 2 days of arrival at the laboratory. The time from harvest to the arrival of spinach at Athens was estimated at two to four days. During transportation, the rosettes of spinach were held together in bunches covered by paper and shipped in waxed fiberboard boxes.

The broccoli was also California grown and purchased from a local distributor. Broccoli was prepared as soon as it was received. The broccoli was received packed in ice in waxed fiberboard boxes.

Sample Preparation

The entire spinach rosette is usually harvested by cutting the tap root at the soil surface. In general, it is not washed before long-distance shipping since doing so might accelerate its decay. The spinach was washed with tap water first to get rid of most of the mud and sand and then with deionized water. The washed spinach was spread out in shallow draining baskets with intermittent stirring to dry uniformly. When the spinach was air-dried back to the point of no visible moisture, it was considered as a raw sample and ready for subsequent processing.

Fresh broccoli is usually harvested by cutting with 8-10 in. (20-25 cm) of stem. It is highly perishable and usually cooled right after harvest at temperatures of 0.6-2.8 °C. Preparation of the broccoli include removal of the leaves and dividing individual flower heads. The small heads of broccoli were washed with tap water and then with deionized water. Like spinach, the broccoli was drained and air dried.

Processing

Blanching. Commercial blanching of spinach is accomplished either by emmersing raw product in water for 3 min at 100 °C or for 6 min at either 85 °C or 100 °C (Lopez 1981). To approximate industrial conditions, an intermediate process was chosen for this study (4 min at 100 °C). To water blanch the vegetables, spinach or broccoli was immersed in boiling water in a steam kettle in a 1:7 ratio, using 4 kg in 28 L water for spinach, and 1 kg in 7 L of water for broccoli. This ratio closely approximates that recommended in USDA Bulletin -10 (Anon. 1974) for the blanching of vegetables. After boiling commenced, the vegetables were blanched for 4 min. The blanched vegetables were cooled immediately by immersion in ice water for 1 min and allowed to drain.

Steam-blanching was carried out by placing a 200 g vegetable sample in a plastic colander on a perforated platform in a home style canner containing 500 mL boiling water. After a 4 min blanch, the vegetables were cooled by immersion in ice water and drained.

Canning. Following the water-blanch treatment, 350 g drained spinach were placed in 500 mL jars with 60 mL deionized water, sealed and processed in a retort at 122 °C for 54 min at 15-18 psi (Lopez 1981). Jars were cooled by water spray and placed in storage in the dark at 21 °C. Samples of spinach as well as the canning medium were analyzed immediately after canning and again after 3 months storage.

Freezing. After water-blanching, 100 g samples of spinach and broccoli were packed in heat-sealed unevacuated freezer pouches, which were stored at −32.2 °C in a blast freezer for 3 months for spinach, and 60 days and 160 days for broccoli.

Samples of spinach and broccoli were analyzed at every processing step for free and total pantothenic acid. Pantothenic acid was measured according to the

microbiologial method of Zook *et al.* (1956) employing a double enzyme digestion by pigeon liver extract and alkaline phosphatase. Free pantothenic acid was determined from sample extracts that were not subjected to enzyme hydrolysis. The microorganism used was *Lactobacillus plantarum* (ATCC 8014). At least four replicate values were obtained from each of two independent trials. One-way analysis of variance and LSD (least signifigant difference) analysis were used to test for significant differences between treatments.

RESULTS AND DISCUSSION

Spinach

Total pantothenic acid (TPA) values for raw spinach, water-blanched spinach and steam-blanched spinach were 0.184 mg/100 g, 0.066 mg/100 g and 0.160 mg/100 g, respectively (Table 1). Percent retention values based on the raw value were 87% for steam-blanched spinach and 36% for water-blanched spinach. The data clearly show the significance of leaching on loss of pantothenic acid from a leafy green vegetable. Retention of free pantothenic acid was less than that noted for TPA indicating that the free vitamin is lost more readily from the leaf structure than the more complex forms of the vitamin. No literature values were available for comparison of blanching effects on pantothenic acid content of spinach.

Canned spinach (after water-blanching) contained 0.053 mg/100 g of TPA. This value represented 29% retention based on raw spinach content. Orr (1969) reported that canned spinach retained 22% of total pantothenic acid. The TPA content of canned spinach stored at room temperature for 90 days was 0.056 mg/100 g which was not significantly different ($\alpha = 0.05$) from that present in canned spinach before storage. No significant change ($\alpha = 0.05$) in TPA was observed in 90 days of frozen storage of water-blanched spinach.

Broccoli

Data on TPA content of broccoli are presented in Table 2. TPA values were 0.934 mg/100 g, 0.610 mg/100 g and 0.358 mg/100 g for raw, steam-blanched broccoli and water-blanched, respectively. As with spinach, steam blanching significantly ($\alpha = 0.05$) improved retention of TPA compared to water-blanching (Table 2). Our retention values were somewhat lower than values reported by Morgan *et al.* (1945) for steam blanched (65% vs. 75%) and water blanched (38% vs. 45%) broccoli.

Free pantothenic acid was retained at higher levels in broccoli than observed in spinach.

A significant loss ($\alpha = 0.05$) of 24% occured during frozen storage of water blanched broccoli at -32.2 C for 60 days. Upon longer storage up to 160 days, no further loss was observed.

TABLE I.
EFFECTS OF PROCESSING ON FREE AND TOTAL PANTOTHENIC ACID (PA) CONTENT OF SPINACH

Processing	Free PA		Total PA	
	mg/100 g	% R*	mg/100 g	% R*
Raw (12)**	0.141[a] ±0.006	-	0.184[a] ±0.016	-
Steam blanched (8)	0.095[b] ±0.007	67	0.160[b] ±0.013	87
Water blanched (8)	0.029[c] ±0.001	21	0.066[c] ±0.003	36
Canned (8)	0.024[d] ±0.006	17	0.053[d] ±0.006	29
Canned/stored (8) (90 days)	0.021[d] ±0.004	15	0.056[d] ±0.003	30
Frozen (8) (90 days)	0.022[d] ±0.001	16	0.064[cd] ±0.003	35

★ Retention values were calculated in relation to raw.
★★ Number of observations
Each value is Mean ± S.D.
Same superscripts in the same column indicate no significant differences ($\alpha = 0.05$).

In conclusion, steam-blanching, water-blanching, canning and frozen storage caused losses of pantothenic acid in spinach and broccoli to different degrees. Water-blanching incurred more loss of pantothenic acid than steam-blanching for both vegetables. Canning of spinach caused a significant loss of pantothenic acid. However, no futher loss occurred during 90 days storage of canned spinach at room temperature. During frozen storage, free pantothenic acid in spinach decreased but total pantothenic acid did not change. A decrease in the pantothenic acid content of water-blanched broccoli was observed during 60 days frozen storage, but the level did not change upon longer storage.

The study provides quantitative information of pantothenic acid loss in spinach and broccoli through several processing steps. The relative role of each step in the loss of the vitamin is indicated. The data define the role of blanching in the loss of pantothenic acid during processing of these vegetables.

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TABLE 2.
EFFECTS OF PROCESSING ON FREE AND TOTAL PANTOTHENIC ACID (PA) CONTENT OF BROCCOLI

Processing	Free PA mg/100 g	Free PA % R*	Total PA mg/100 g	Total PA % R*
Raw (8, 8)**	0.492[a] ±0.047	-	0.934a ±0.039	-
Steam blanched (8, 8)	0.378[b] ±0.044	77	0.610[b] ±0.029	65
Water blanched (8, 8)	0.071[c] ±0.023	35	0.358[c] ±0.023	38
Frozen (16, 8) (60 days)	0.127[d] ±0.012	26	0.271[d] ±0.014	29
Frozen (8, 24) (160 days)	0.127[d] ±0.022	26	0.242[d] ±0.067	26

★ Retention values were calculated in relation to raw.
★★ Number of observations for free and total pantothenic acid, respectively.
Each value is Mean ± S.D.
Same superscripts in the same column indicate no significant differences ($\alpha = 0.05$)

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EFFECT OF COOKING TEMPERATURE AND VARIATIONS IN SWEETENED CONDENSED WHOLE MILK ON TEXTURAL PROPERTIES OF CARAMEL CONFECTIONS

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ABSTRACT

The cooking temperature of the caramel mix, and processing conditions used for sweetened condensed whole milk (SCWM), including time and temperature of the forewarming treatment, were factors having the greatest effect on textural properties of caramel. Caramel texture was not affected when calcium levels in milk used in the formulation, were varied in the range of 20% above to 20% below the average value. The microbiological quality and acidity of SCWM had very little effect on caramel properties.

INTRODUCTION

Variations in caramel texture have been attributed to variations in milk ingredients used in caramel formulations. Sweetened condensed milk (full cream or skim), evaporated milk, or nonfat dry milk are the milk ingredients of choice for producing superior caramel (Smith 1978). Dried or condensed whey and caseinates have been used with only limited success because of excess browning during cooking and poor texture of the caramel product (DeBruin and Keeney 1969; Kinsella 1970; Lees and Jackson 1975; Minifie 1975; Pyrz 1976).

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Seasonal variations in raw milk were the focus of attention in two studies (Weckel and Steinke 1973; Macallister 1976). Variations in caramel flow properties did occur over three and twelve month periods, however, no correlation was established between these variations and the well known seasonal variations in milk.

The viscosity of caramel increased steadily with increased amounts of milk solids in the formulation (Duck 1959; Weckel and Steinke 1973).

Varying the temperature of the pretreatment given to milk before condensing (forewarming) affected caramel flow properties (Weckel and Steinke 1973). Samples of sweetened condensed skim milk (SCSM) were forewarmed at 145, 165, 185, and 205°F. (62.7, 73.9, 85.0, 96.1°C) for 30 min each. The diameter of a 25 ± 0.4-g sample of deposited caramel decreased steadily as the forewarming temperature increased. It was also found that if the pH of SCSM was decreased with lactic acid in the range of 6.2 to 4.7, the viscosity of caramel decreased and the diameter of the deposited caramel increased.

The textural properties of caramel could be affected by changes in milk protein caused by proteolytic enzymes. Psychrotrophic microorganisms isolated from raw milk, multiply at or below 45°F (7.2°C) and produce heat stable proteolytic enzymes (Cousin and Marth 1977; Law 1979; Gebre-Egziabher *et al.* 1980; Patel *et al.* 1983). Although no data is available on the effect on caramel properties, these enzymes have been associated with increases in protein breakdown in stored milk and losses in yield as well as changes in texture of cheese (Cousin and Marth 1977; DeBeukelar *et al.* 1977).

Small changes in milk salt composition can have great effects on protein stability and the stability of milk products (Pyne 1962), possibly affecting caramel texture. The average amount of calcium in skim milk is 120 mg/100 mL or approximately 0.12%. The reported range is 100 to 140 mg/100 mL or approximetely ± 20% from the average value. Extremes have been reported however, from 65 to 264 mg/100 mL (Jenness and Patton 1959).

The objective of this study was to identify variations in sweetened condensed whole milk (SCWM) that had a measurable effect on the textural properties of caramel, and if possible, recommend procedures for producing SCWM that would minimize variability in caramel texture. Variations in SCWM included acidity, forewarming, microbiological quality and salt composition.

MATERIALS AND METHODS

Caramel Formulation

The caramel formulation used throughout this study is shown in Table 1. It was reviewed and approved by members of the National Confectioners Association (NCA) and was considered to be representative of typical formulations used in the industry.

TABLE 1.
CARAMEL FORMULATION

Ingredient	%
Corn syrup, 42 DE	39.9
Sweetened condensed whole milk (SCWM)	34.9
Sucrose	14.9
Coconut oil	9.9
Salt (NaCl)	0.3
Soy lecithin	0.1

Caramel Preparation

Caramel in 23-kg lots was prepared in the pilot plant in a Savage kettle (S-48 firemixer). The ingredients were premixed over low heat until smooth. The mix was cooked over a medium flame at a mixing speed of 56 RPM until the temperature reached 242 ± 1°F (116.7 ± 0.6°C), as determined by a type T thermocouple. The time required to reach this temperature was 25 ± 1 min.

Laboratory samples of 3.5 kg were prepared as follows: Fat (Durkee Hydrol 92, partially hydrogenated coconut oil) and emulsifier (Lecithin, BF #581), Panaplus) were melted together at 176°F (80.0°C), in a stainless steel mixing bowl. The temperature was allowed to drop to just above the melting range of the fat 95 to 104°F (35.0 to 40.0°C) at which time SCWM (Galloway Company, or SCWM prepared in the laboratory) and corn syrup (A. E. Staley Manufacturing Company) were added. The mixture was blended until smooth (2 min) using a Hobart mixer set on low speed (68 RPM). Sugar and salt were added slowly and mixing continued for another 15 min on medium speed (112 RPM).

Samples of 1.1 kg from the premixed lot were transferred to a small kettle for cooking. The kettle was equipped with a type T thermocouple placed near the bottom for monitoring temperature. Placement of the tip toward the center of the caramel mass was critical to achieve consistent results.

Teflon mixing paddles, designed to continuously scrape hot caramel from the sides of the kettle, were driven by a motor and revolved at 35 RPM. The caramel mix was cooked until it reached the final temperature of 242 ± 1°F (116.7 ± 0.6°C). The heat source was a 350°F (176.7°C) constant temperature oil bath (Silicone bath fluid, 6428-R17, The Arthur H. Thomas Company). The temperature of the oil bath was adjusted to provide a cooking time of 25 ± 1 min, similar to that required for pilot plant samples. Heating curves of time versus temperature were made for both pilot plant and laboratory samples for the purpose of comparison (Fig. 1).

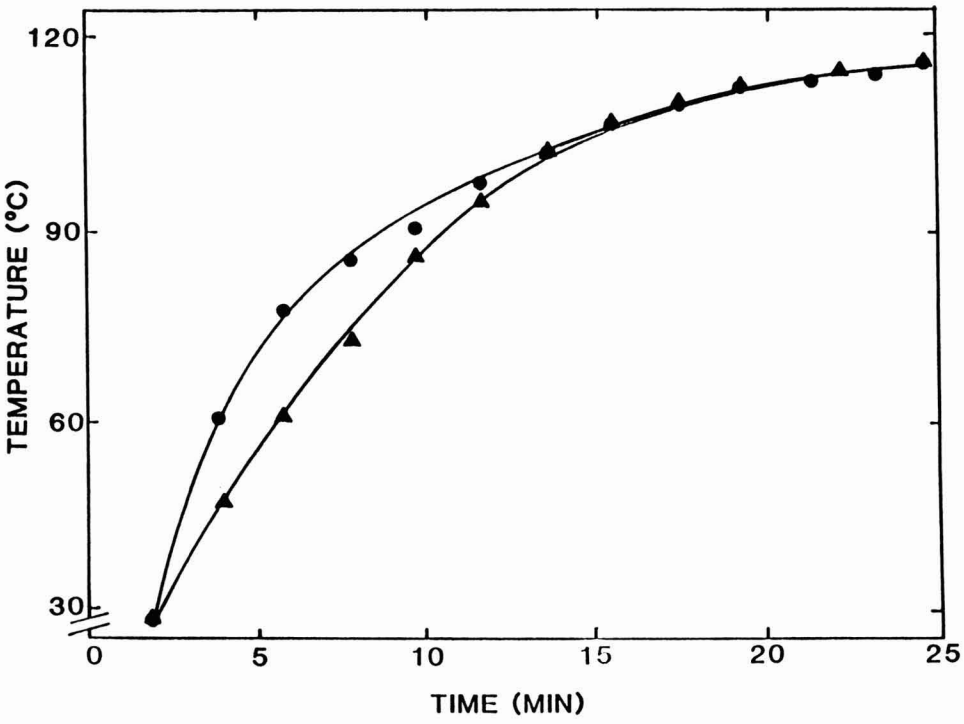

FIG.1. HEATING CURVES OBTAINED DURING COOKING OF 23-KG AND 1100-G LOTS OF CARAMEL
Error bars indicate one standard deviation from the mean.
▲ 23-kg lot; ● 1100-g lot

The mix was poured into either teflon molds or a rectangular form on a water cooled steel table. Cooled samples were wrapped in moisture-proof paper and stored at 72°F (22.2°C) for later use.

Sample Analysis

Apparent viscosity of the hot caramel mix was determined after the temperature of the mix had dropped to 240°F (115.6°C). The time needed for the cooked caramel to reach this temperature was 2 min ± 20 s. A Brookfield Synchro-Lectric viscometer (model RVF) set at 10 RPM was used. The spindle chosen was either size 4 or 5 depending on the sample viscosity. A reading was taken after three revolutions of the spindle.

The moisture content of caramel was determined using caramel from the center of the large rectangular sample after 24 h storage at 72°F (22.2°C). For each moisture determination, duplicate two-gram samples, consisting of very small caramel pieces, were dissolved in double distilled water. Celite was added to in-

crease the surface area during drying. The samples were dried under vacuum at 158°F (70.0°C) for 18 h.

The pH of caramel was determined after dissolving a five-gram sample of finely cut caramel pieces in 50 mL of double distilled water in a 125-mL Erlenmeyer flask. The pH readings were taken using an Orion pH meter (model 601A) equipped with a glass electrode.

Caramel hardness (force needed for 80% compression) was measured on an Instron Universal Testing Machine, Model 1132. Cylindrical caramel pieces were stored in teflon molds at 72°F (22.2°C) in an incubator for 24 h. The molds were then stored for less than one hour in an insulated container until immediately before measuring the hardness. The caramel pieces measured 19 mm in diameter by 19 mm in height. They were compressed to 80% of their original height between two parallel plates. A load cell was used which had a maximum capacity of 1000 lb. The rate of compression was 5.08 cm per minute and the chart speed was 50.80 cm/min. The chart was calibrated using an electrical calibration equivalent to 500 lb. The height of each compression peak indicated the hardness of caramel or the force needed for 80% compression.

SCWM Preparation: Commercial Versus Laboratory

Commercially prepared SCWM (Galloway Company, Neenah, WI) was used in experiments to determine the effect of cooking temperature and SCWM acidity on caramel properties. Milk was pasteurized at 190 to 210°F (87.8 to 98.9°C) in a high temperature short time single pass pasteurizer, then evaporated in a continuous triple effect rising film evaporator at temperatures in the three stages of 185, 165, and 145°F (85.0, 73.9, and 62.8°C), respectively. The average overall dwell time in the evaporator was 1.5 h. The solids content was 71% as measured on a Bausch & Lomb Abbe refractometer.

Alternatively, SCWM was prepared in the laboratory for use in experiments to determine the effect of the severity of forewarming, microbiological quality, and salt composition of milk, on caramel properties. For these experiments the use of commercially prepared SCWM was not appropriate because the raw milk had to be subjected to each treatment before evaporation, to evaluate the effect. Raw milk was obtained from the University of Wisconsin-Madison dairy plant and standardized to 4% fat by the addition of cream. It was batch pasteurized and forewarmed under various conditions depending on the experiment, then rapidly cooled over a surface heat exchanger. Sugar was added to achieve a final concentration of 42% and the sweetened milk was condensed in a laboratory vacuum distillation apparatus to 71% solids as measured with a Bausch & Lomb Abbe refractometer.

The apparent viscosity of SCWM was measured at 72°F (22.2°C) with a Brookfield Synchro-Lectric viscometer, model RVF, at 10 RPM, using a size 3 spindle.

Cooking Temperature

The final cooking temperature of the caramel mix was varied in the range of 239 to 245°F (115.0 to 118.3°C).

SCWM Acidity

The pH of SCWM was varied in the range of 6.7 (control value) to 5.8 by adding lactic acid after the milk had been concentrated.

Forewarming of Milk

Four sublots of milk were forewarmed at 145, 165, 175 (control) and 185°F (62.7, 73.8, 79.4, and 85.0°C), each for a constant time of 20 min.

Microbiological Quality of Milk

Raw milk was held at 45°F (7.2°C) for 6 days. In another experiment, raw milk was inoculated with 10^3 psychrotrophs/mL and held at 45°F (7.2°C) for 6 days. Fresh milk, immediately pasteurized and forewarmed, was used as the control. Samples were batch pasteurized and forewarmed at 175°F (79.4°C) for 20 min and condensed as described above.

Salt Composition of Milk

The calcium content of milk was increased by adding calcium chloride ($CaCl_2$), and decreased by chelating calcium with ethylenediaminetetraacetic acid (Na_4 EDTA). Calcium levels were varied by 10, 20, and 50% above and below the average calcium level. Milk was pasteurized and forewarmed at 175°F (79.4°C) for 20 min and condensed as described above.

RESULTS AND DISCUSSION

Cooking Temperature

It is well known that the final cooking temperature of caramel affects the moisture content and therefore the textural properties of caramel (Minifie 1980; Lees and Jackson 1975). In this study, the data obtained from textural measurements (apparent viscosity and hardness) of caramel cooked to various temperatures, were examined to determine if the measurements were appropriate for detecting differences in caramel texture. The moisture content decreased from 10.15 to 9.02% as the cooking temperature of caramel increased from 239 to 245°F (115.0 to 118.3°C) (Fig. 2). Apparent viscosity and hardness values increased from 130 to 176 poise and from 44 to 131 lb, respectively. The variation in values confirmed that the methods used to measure textural properties were appropriate.

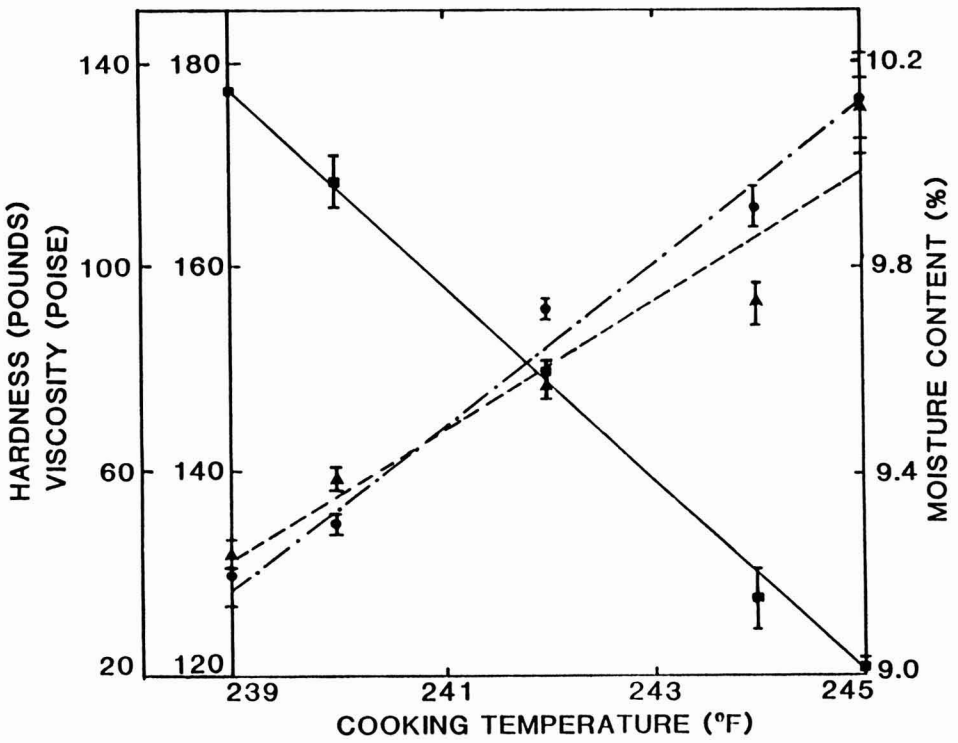

FIG. 2. EFFECT OF COOKING TEMPERATURE ON HARDNESS, VISCOSITY, AND MOISTURE CONTENT OF CARAMEL

Error bars indicate one standard deviation from the mean.

▲----▲Hardness
●—•—● Viscosity
■———■ Moisture content

The data also show that great changes in moisture content and texture of caramel were brought about by a 2 °F (1.1 °C) variation in cooking temperature. These results are in agreement with those reported by Weckel and Steinke (1973). This reemphasized the fact that the cooking temperature is very important and must be strictly controlled to produce caramel of uniform texture.

Acidity of SCWM

The pH of condensed milk may vary slightly depending on the amount of free acid present at the time milk is condensed and the amount of heat applied during processing. There was a very small decrease in apparent viscosity and hardness of caramel from 158 to 148 poise and 77 to 75 lb, respectively, as the pH of SCWM (pH 6.70) was decreased to 5.80 (Table 2). This is in agreement with results reported by Weckel and Steinke (1973) when SCSM within this pH range

TABLE 2.
EFFECTS OF SCWM ACIDITY ON VISCOSITY, CARAMEL VISCOSITY, MOISTURE CONTENT, pH, AND HARDNESS

SCWM[a]		Caramel[b]			
pH	Apparent viscosity poise	Apparent viscosity poise	Moisture content w/w%	pH	Hardness lbs
6.70 ± .01 (control)	53.8 ± .6	158 ± 3	9.30 ± .01	6.25 ± .01	77 ± 5
6.30 ± .01	55.5 ± .1	154 ± 3	9.24 ± .07	6.31 ± .04	75 ± 7
5.80 ± .01	53.6 ± .7	148 ± 4	9.51 ± .01	6.24 ± .03	75 ± 7

[a]SCWM was obtained from the Galloway Company, Neenah, WI.
[b]Cooking temperature was 242 ± 1°F for all samples.
Each value represents the mean ± s. d. of duplicate determinations.

was used, and suggests that sweetened condensed milk may be slightly acidic with little effect on textural properties of caramel. Separation of SCWM occurred at pH values less than 5.80, therefore, these samples were not considered appropriate for use in caramel formulations.

Severity of the Forewarming Treatment

The apparent viscosities of SCWM and caramel increased as the forewarming temperature was increased in the range of 145 to 185°F (62.7 to 85.0°C) (Fig. 3). Hardness also increased with the largest increases occurring at temperatures greater than 175°F (79.4°C). Extensive denaturation and crosslinking of milk protein presumably occurred at these temperatures. This could have caused the large increase in SCWM viscosity which then affected the textural properties of caramel.

A relationship between the apparent viscosity of SCWM and textural properties of caramel is seen in Fig. 4. The viscosity and hardness of caramel increased as the viscosity of SCWM increased in the range of 40 to 75 poise. The greatest increases occurred at SCWM viscosity values greater than 56 poise. This result illustrates the importance of using SCWM lots having like viscosity values to produce caramel of uniform texture.

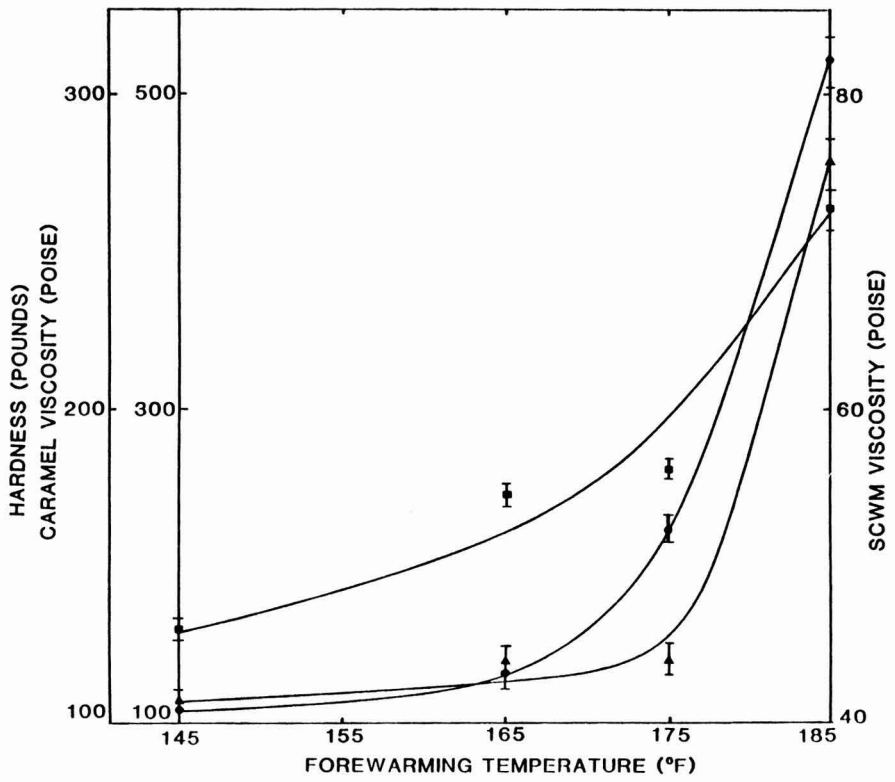

FIG. 3. EFFECT OF FOREWARMING TEMPERATURE ON SCWM VISCOSITY, CARAMEL VISCOSITY, AND HARDNESS

Error bars indicate one standard deviation from the mean.

■ SCWM viscosity;
● Caramel viscosity;
▲ Hardness

Variations in the Microbiological Quality of Milk

The data in Table 3 show the effects on caramel properties when SCWM was used that was produced from milk with high psychrotroph populations as might be the case if milk were held for several days before pasteurization. The data also show the effect when SCWM was used that was produced from milk that was inoculated with 10^3 psychrotrophs/mL to reach larger than normal counts (10^7/mL) as might be the case if milk were contaminated before pasteurization. In both cases there was a slight decrease in apparent viscosity and hardness of caramel with increasing numbers of psychrotrophs. There was little variation in apparent viscosity of SCWM, moisture content and pH of caramel. The results suggest that the textural properties of caramel are not affected by psychrotrophic microorganisms or the enzymes they produce in milk.

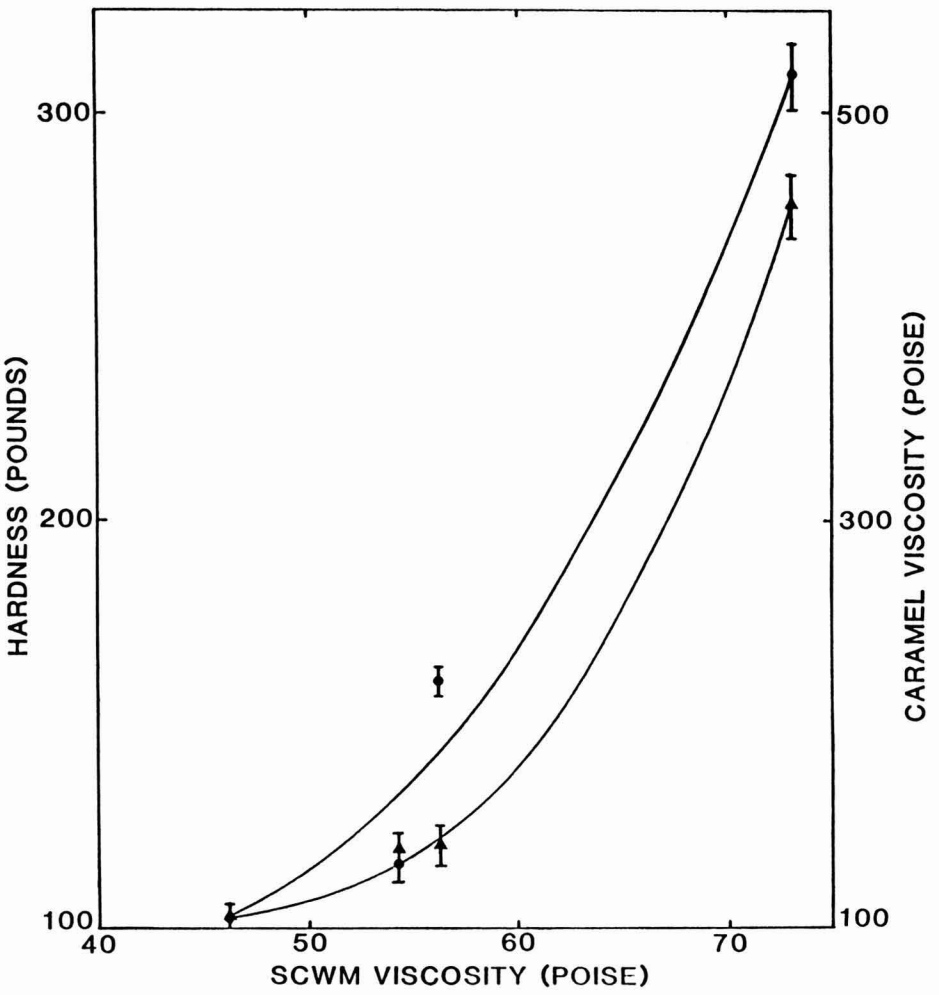

FIG. 4. RELATIONSHIP BETWEEN SCWM VISCOSITY, CARAMEL VISCOSITY, AND HARDNESS

Error bars indicate one standard deviation from the mean.

● Caramel viscosity;
▲ Hardness

Effect of Variations in Milk Salt Composition

SCWM was produced from milk containing varying amounts of calcium and was used in the caramel formulation to study the effect on caramel properties. The results are shown in Table 4. The apparent viscosity of SCWM decreased as the calcium content increased. Textural properties of caramel were not affected

TABLE 3.
EFFECTS OF PSYCHROTROPH POPULATIONS IN MILK ON SCWM VISCOSITY, CARAMEL VISCOSITY, MOISTURE CONTENT, pH, AND HARDNESS

Milk[a]		SCWM	Caramel[b]			
Age days	Psychrotrophs cfu/ml	Apparent viscosity poise	Apparent viscosity poise	Moisture content w/w%	pH	Hardness lbs
Not Inoculated						
1	< 100	53.4 ± .4	237 ± 9	9.19 ± .03	6.29 ± .03	129 ± 4
6	2.4×10^6 $\pm 1 \times 10^5$	54.8 ± .3	216 ± 7	9.26 ± .07	6.26 ± .08	122 ± 3
Inoculated						
1	4.0×10^3 $\pm 4 \times 10^2$	55.3 ± .4	215 ± 4	9.32 ± .01	6.24 ± .03	121 ± 4
3	9.6×10^6 $\pm 4 \times 10^5$	53.1 ± .3	195 ± 7	9.39 ± .03	6.31 ± .01	116 ± 1
6	3.6×10^7 $\pm 9 \times 10^6$	56.0 ± .3	184 ± 3	9.28 ± .03	6.21 ± .03	113 ± 1

[a]Milk was obtained from the University of Wisconsin-Madison dairy plant.
[b]Cooking temperature was 242 ± 1°F for all samples.

Each value represents the mean ± s.d. of duplicate determinations

in the normal range of 20% above to 20% below the average value. Alternatively, changes in calcium of 50% above or below the average value had a great effect. Apparent viscosity and hardness of caramel were 44 poise and 42 lb, respectively, at a calcium level 50% above the average, and 327 poise and 204 lb, respectively, at a calcium level 50% below the average. These results suggest that a slight variation in calcium that could occur in pooled milk should not be of concern, because calcium levels would not be expected to vary by more than ± 20% and there were no measureable effects on caramel properties in that range.

Effect of Processing Conditions of SCWM

Control values from each set of experimental data discussed above were tabulated to illustrate the effects of SCWM processing on caramel properties (Table

TABLE 4.
EFFECTS OF THE CALCIUM CONTENT OF MILK ON SCWM VISCOSITY, CARAMEL VISCOSITY, MOISTURE CONTENT, pH, AND HARDNESS

Milk[a]	SCWM	Caramel[b]			
Calcium content relative to control	Apparent viscosity poise	Apparent viscosity poise	Moisture content w/w%	pH	Hardness lbs
+50%	30.0 ± .1	44 ± 4	9.29 ± .03	6.31 ± .04	42 ± 1
+20%	40.4 ± .3	224 ± 6	9.19 ± .06	6.30 ± .01	134 ± 1
+10%	47.8 ± .3	238 ± 4	9.20 ± .03	6.31 ± .01	129 ± 1
control	55.5 ± .1	232 ± 6	9.25 ± .05	6.22 ± .01	132 ± 1
-10%	63.5 ± .1	242 ± 4	9.12 ± .04	6.20 ± .04	132 ± 4
-20%	71.5 ± .1	244 ± 4	9.29 ± .04	6.28 ± .02	134 ± 1
-50%	90.3 ± .2	327 ± 3	9.16 ± .03	6.28 + .02	204 ± 1

[a]Milk was obtained from the University of Wisconsin-Madison dairy plant.
[b]Cooking temperature was 242 ± 1°F for all samples.

Each value represents the mean ± s.d. of duplicate determinations

5). SCWM viscosity values were similar (55 poise) for both commercial and laboratory processed milk. Alternatively, the hardness values were approximately 77 and 127 lb using commercial and laboratory processing, respectively. Caramel viscosity values were approximately 157 and 226 poise. These results suggest that the processing method used for SCWM had an effect on caramel texture even though it did not affect SCWM viscosity.

Recommendations

It is strongly recommended that the cooking temperature and time be carefully controlled to produce caramel of consistent texture.

SCWM viscosity may be specified by the caramel manufacturer, to the milk supplier, to achieve the desired texture of caramel if the processing method is the same as it was for lots of milk used previously. If, however, the process has changed, variations in caramel texture may be noticed even if milk viscosities are the same. It is recommended, therefore, that SCWM specifications include both processing conditions and viscosity.

TABLE 5.
EFFECT OF PROCESSING CONDITIONS OF SCWM ON VISCOSITY, CARAMEL VISCOSITY, MOISTURE CONTENT, pH, AND HARDNESS

SCWM		Caramel[a]			
Process[b]	Apparent viscosity poise	Apparent viscosity poise	Moisture content w/w%	pH	Hardness lbs
A	55.4 ± .1	156 ± 1	9.60 ± .01	6.25 ± .04	77 ± 4
A	53.8 ± .6	158 ± 3	9.30 ± .01	6.25 ± .01	77 ± 7
B	53.4 ± .4	237 ± 8	9.19 ± .03	6.29 ± .03	129 ± 4
B	55.3 ± .4	215 ± 4	9.32 ± .01	6.24 ± .03	121 ± 4
B	55.5 ± .1	232 ± 8	9.25 ± .07	6.22 ± .01	132 ± 1

[a]Cooking temperature was 242 ± 1°F for all samples.
[b]A: commercially processed (Galloway Company, Neenah, WI)
B: laboratory processed (University of Wisconsin, Madison, WI)

Each value represents the mean ± s.d. of duplicate determinations

ACKNOWLEDGMENTS

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BASE CATALYZED TRANSESTERIFICATION OF VEGETABLE OILS[1]

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ABSTRACT

Anhydrous methanolic sodium hydroxide catalyzed transesterification of vegetable oils yielded pure methyl esters within one minute at ambient temperature under nitrogen. Neither benzene nor phenolphthalein was required to maintain adequate stability of methanolic sodium hydroxide transesterification reagent. Additional esterification with methanolic HCl to convert free fatty acids to methyl esters was not required. Neutralization of excess base with 2.0 M HCl stopped saponification. Transesterification catalyzed by 1.0 M methanolic NaOH was reproducible and quantitative. Pentane and diethyl ether were excellent solvents for methanolic sodium hydroxide catalysis.

INTRODUCTION

Base catalyzed transesterification in methanol, alternatively called alcoholysis or transmethylation, is a widely used procedure for preparation of fatty acid methyl esters (FAME) of triglycerides, fats and other lipids. Bases used in transesterification include: sodium methoxide or potassium hydroxide in methanol (Christopherson and Glass 1969); sodium methoxide in methanol/methyl acetate (Christie 1982); sodium methoxide, 2, 2-dimethoxypropane (DMP) and methanol/benzene (Mason *et al.* 1964); metallic sodium in anhydrous methanol/benzene-phenolphthalein, or sodium hydroxide in anhydrous methanol/benzene-phenolphthalein

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(Glass 1971). Sodium hydroxide or methoxide in methanol at 60°C (Freedman *et al.* 1984) provide alkaline catalysts for transesterification of vegetable oils.

Saponification followed by reesterification using methanolic sodium hydroxide for methyl ester preparation (Metcalfe *et al.* 1966) required heating and addition of methanolic BF_3 or HCl to drive esterification to completion.

Many transesterification methods use potentially hazardous reagents and solvents such as benzene (Glass 1971), and are not suitable for food application such as the preparation of sucrose polyesters (SPE). With the recent FDA approval for use of vegetable oil methyl esters in the preparation of sucrose esters, a new methylation reagent devoid of potentially hazardous solvents is highly desirable. The objectives of this investigation were to develop a stable methanolysis reagent or catalyst, and a transesterification procedure suitable for food application and GLC analysis; to develop a methylation procedure providing quantitative conversion of fatty acids to methyl esters under sufficiently anhydrous conditions; to explore solvents other than benzene for transesterification; and to establish a methylation procedure avoiding the use of heat and additional reagents such as methanolic HCl or BF_3 to catalyze reesterification.

MATERIALS AND METHODS

Pure vegetable oils of soybean, safflower, corn, peanut and cottonseed were purchased locally (Pullman, WA). Tripalmitin, triolein, trilinolein and trilinolenin (99% pure) were purchased from Sigma Chemical Co. (St. Louis, MO). Sulfuric acid-dichromate solution (50% H_2SO_4), fatty acid methyl esters and mixed methyl ester standards were purchased from Supleco, Inc. (Bellefonte, PA). Sodium hydroxide (ACS grade), anhydrous diethyl ether, pentane, anhydrous sodium and magnesium sulfate, and methanol (HPLC grade) were purchased from J. T. Baker Co. (Phillipsburg, NJ). Molecular sieves 4A were purchased from Aldrich Chemical Co. (Milwaukee, WI).

Reagents

Anhydrous methanolic sodium hydroxide, 1.0 M, was prepared by dissolving 4.0 g sodium hydroxide pellets in 100 mL methanol (HPLC grade), previously dried overnight over molecular sieve 4A. The 1.0 M methanolic sodium hydroxide was stored under anhydrous conditions at room temperature (ca. 22°C).

Thin-Layer Chromatography

Supelco Redi-coats G Silica gel 20 × 20 cm plates (Supelco Inc., Bellefonte, PA) 0.0250 mm thick were activated at 110°C for 30 min and cooled to room temperature. The developing solvent system was petroleum ether:diethyl ether:acetic acid 90:10:1 (v/v/v). Developed TLC plates were sprayed with sulfuric

acid-dichromate (50% H_3SO_4) solution and methyl esters visualized as light brown to black spots after heating at 100–105 °C for 5–10 min.

Gas Liquid Chromatography (GLC)

Methyl esters were injected (on column) into a Hewlett-Packard 5880A gas liquid chromatograph (Avondale, PA) equipped with a flame ionization detector (FID). The GLC column used was a fused silica capillary Supelcowax 10, 60 m × 0.32 mm ID (Supelco Inc., Bellefonte, PA). The following conditions were used to separate fatty acid methyl esters: Column temperature was programmed from 160 °C initial time of 2.5 min to 220 °C with a rate of 3 °C/min. The final temperature was held for 25 min. Detector temperature was 250 °C. The flow rates were: nitrogen, 33.3 mL/min; hydrogen, 30 mL/min; helium, 1.7 mL/min; and air, 400 mL/min. The chart speed was 0.5 cm/min.

Fatty acid methyl esters were quantified by an on-line Hewlett-Packard 5880A electronic integrator (Avondale, PA), and identified by comparing retention times with standards. The GLC conditions were optimized using fatty acid methyl ester standards having chain length distributions simulating fatty acid chain lengths common to vegetable oils.

Base (1.0 M methanolic NaOH) Catalysis

Two hundred milligrams of the vegetable oils were dissolved in a dry screw cap vial containing 1.0 mL pentane or anhydrous diethyl ether. Modification of the initial transesterification reagent of Glass (1971) includes removal of benzene and phenolphthalein. Three to five milliliters of 1.0 M anhydrous methanolic sodium hydroxide was added. The vial was capped and the solution mixed by vortexing vigorously for 1 min. Transesterification was stopped by addition of 2.0 M HCl and pH adjusted to neutrality using pH indicator paper. For time course studies, an aliquot of twenty to thirty microliters was withdrawn at one minute intervals, neutralized with 2.0 M HCl and five to ten microliters spotted directly on a TLC plate. All aliquots for TLC analysis were neutralized with 2.0 M HCl. All reactions were carried out at ambient temperature (ca. 22 °C) under nitrogen.

GLC analysis of fatty acid methyl esters required neutralization of excess NaOH with 2.0 M HCl to protect the GLC column, and to prevent hydrolysis during subsequent storage. Additional 1.5 to 2 mL pentane or anhydrous ether was added and the mixture centrifuged. A one microliter aliquot of the organic layer containing fatty acid methyl esters was diluted with 1 mL pentane, and one microliter was injected into the capillary GLC. The remaining organic layer was dried over anhydrous magnesium sulfate or anhydrous sodium sulfate, filtered and refrigerated. The aqueous layer containing glycerol and NaCl was discarded.

No further extraction or purification of fatty acid methyl ester was required. Further esterification with methanolic HCl was not necessary since 2.0 M HCl was sufficient to stop saponification from occurring when transesterification was complete.

For large scale preparation of vegetable oil FAME, about 100-1000 g of vegetable oil was dissolved in a large dry Erlenmeyer flask equipped with a mechanical stirrer containing 100-800 mL of pentane or anhydrous diethyl ether. Three to four volumes of 1.0 M methanolic NaOH (by weight of vegetable oil) was added and transesterification carried out as described under nitrogen for 5-10 min with vigorous stirring. Large scale preparation may require longer time to clean-up FAME when compared to small scale preparation. Pentane or ether was removed by rotary evaporation to yield FAME. This method has been used to prepare FAME for use in the synthesis of carbohydrate fatty acid polyesters in our laboratory.

In order to obtain quantitative information, equal weights (50 mg) of tripalmitin, triolein, trilinolein, and trilinolenin were accurately weighed and transesterification carried out with 1.0 M methanolic NaOH in the same way as that used for the vegetable oils. The conventional transesterification involving saponification followed by esterification with BF_3-methanol (Metcalfe *et al.* 1966) was also used to transesterify tripalmitin, triolein, trilinolein, and trilinolenin. The concentrations of FAME derived from transesterification of soybean oil with 1.0 M methanolic NaOH was compared to concentrations of FAME derived from soybean oil with conventional transesterification (Metcalfe *et al.* 1966).

RESULTS AND DISCUSSION

Base Catalyzed Transesterification

Results of 1.0 M methanolic sodium hydroxide catalyzed transesterification time course studies in diethyl ether at ambient temperature under nitrogen atmosphere are presented in Fig. 1. Fatty acid methyl esters of soybean, safflower and cottonseed oil were formed within 1–2 min. Transesterification as observed by TLC was complete with total conversion, based on the disappearance of the starting material transesterified to methyl esters. Heating was not required for fatty acid methyl ester preparation contrary to reports of Freedman *et al.* (1984) and Metcalfe *et al.* (1966). Alcoholysis at room temperature was described by Christopherson and Glass (1969). No free fatty acids remained throughout time course studies as determined by TLC.

Methanolysis was the primary reaction occurring when anhydrous methanolic sodium hydroxide catalyzed transesterification as evidenced by a single spot on TLC (Fig. 1). Methyl esters formed were pure, as determined by TLC and GLC. Anhydrous sodium methoxide (CH_3ONa) generated by dissolving sodium hydroxide in molecular sieve dried methanol is present in large concentration, driving the reaction towards methyl ester formation. Glass (1971) reported that sodium hydroxide in methanol results in essentially all the base present as alkoxide (CH_3ONa) and that esterification will proceed nearly to completion because of the high concentration of the alkoxide formed by excess methanol.

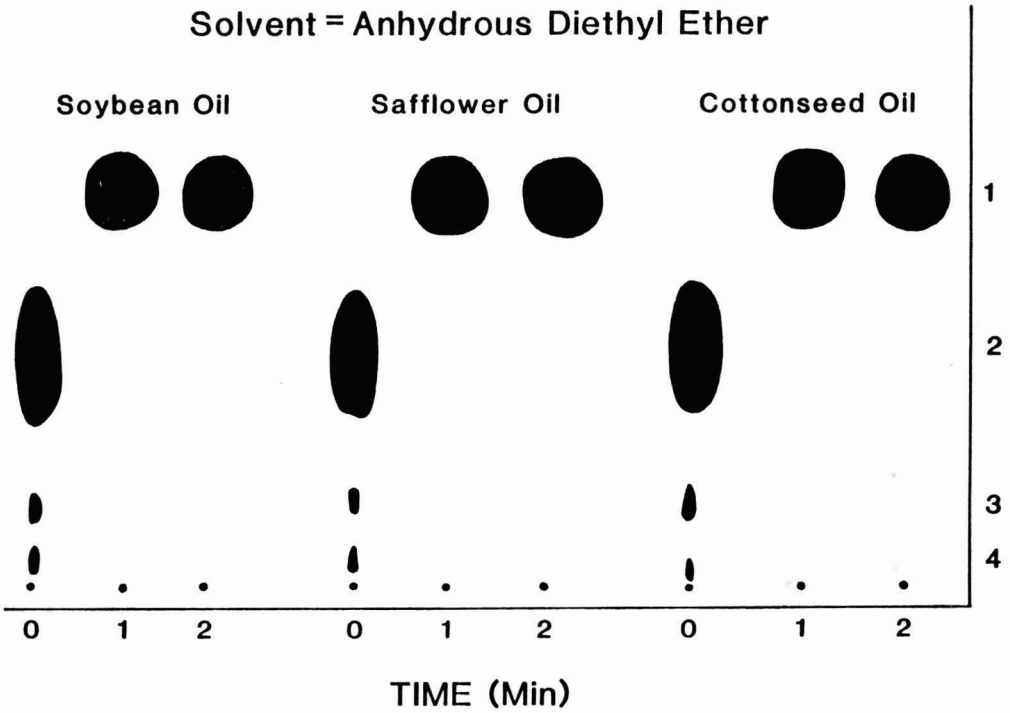

FIG. 1. TRANSESTERIFICATION TIME COURSE STUDIES OF SOYBEAN, SAFFLOWER AND COTTONSEED OIL CATALYZED BY 1.0 M ANHYDROUS METHANOLIC NaOH AT AMBIENT TEMPERATURE UNDER NITROGEN IN DIETHYL ETHER AS DETERMINED BY TLC.
1, methyl esters; 2, triglyceride; 3, diglyceride; 4, impurites. Developing solvent = Petroleum ether: Diethyl ether: Acetic acid, 90:10:1 v/v/v.

Transesterification reported here differs from the conclusions of Glass (1971) and Christie (1982), that trace amounts of free fatty acids were formed and required methanolic BF_3 or methanolic HCl to carry esterification to completion, or addition of methyl acetate to reduce saponification. Freedman *et al.* (1984) and Morrison and Smith (1964) demonstrated that anhydrous conditions inhibited formation of free fatty acids. Freedman *et al.* (1984) also reported that the presence of free fatty acids in oils cause problems in esterification. Moisture is probably the cause of free fatty acid formation during transesterification and anhydrous conditions must be maintained. Christie (1982) observed using the method of Christopherson and Glass (1969) that trace amounts of water taken up from the atmosphere or adsorbed on glassware generated sufficient sodium hydroxide to bring about saponification of polar lipids. Transesterification was carried out in dry test tubes under nitrogen atmosphere to exclude moisture and carbon dioxide, and to prevent oxidation of vegetable oils. The 1.0 M methanolic sodium hydroxide catalyzed transesterification required no methanolic BF_3 or methanolic

HCl to drive esterification to completion. Addition of 2.0 M HCl neutralized excess base, prolonged the life of the GLC column, produced no artifacts in the chromatogram and gave stable methyl esters on storage without saponification.

Neutralization with 2.0 M HCl did not produce detectable amounts of free fatty acids on TLC. It is possible that some sodium salts of fatty acids may be formed during transesterification which will be lost during the clean-up. However, this loss will not affect the total fatty acid profile of the vegetable oils and the validity of the method. Formation of sodium-glycerol derivative (sodium glyceroxide) by the anhydrous methanolic sodium hydroxide may be more favorable than formation of sodium salt, which upon subsequent neutralization with 2.0 M HCl liberated glycerol and NaCl. Saponification will not occur to any appreciable extent because of the low concentration of hydroxide and the rapidity with which transesterification was completed (Glass 1971). Substantially anhydrous oils and reagents were used for the transesterification. This method is not suitable for esterification of free fatty acids present in the original vegetable oils.

Rapid transesterification of partially autoxidized vegetable oils may result in conjugated products (Morrison and Smith 1964). Transesterification reported here was carried out under nitrogen to exclude moisture, carbon dioxide and to prevent autoxidation. Isomerization of unsaturated fatty acids may occur as side reactions under the mild conditions employed for transesterification. Freedman *et al.* (1984) noted that such isomerizations are not significant. The formation of polymers and other side reactions reported by Morrison and Smith (1964) and Freedman *et al.* (1984) were not studied. However, no polymers were detected in methanolic sodium hydroxide when stored under anhydrous conditions.

Bannon *et al.* (1985) demonstrated that neutralization with aqueous hydrochloric acid was effective in stopping the saponification of methyl butyrate, and gave a reaction mixture composition that did not change during storage for 24 h. Bannon *et al.* (1985) recommended a change in the international procedures for methyl ester preparation, and noted that saponification can be stopped by neutralizing the catalyst with hydrochloric acid when the methanolysis is complete. Stable reaction mixtures of methyl esters and highly accurate and reproducible quantitative results were obtained when neutralized with aqueous HCl.

No extraction of fatty acid methyl esters or further purification was required before GLC analysis, thereby minimizing loss of methyl esters, contrary to the observations of Metcalfe and Wang (1981). A typical GLC chromatogram of corn oil methyl esters is presented in Fig. 2. No artifacts were observed near the solvent peak. Christie (1982) and Bannon *et al.* (1985) observed artifact peaks in chromatograms when sodium methoxide or potassium hydroxide was used for transesterification. Neutralization with 2.0 M HCl removed excess base and is comparable to reports of Bannon *et al.* (1985).

Transesterification of vegetable oils catalyzed by 1.0 M methanolic NaOH is reproducible and quantitative with conversion of triglycerides to fatty acid methyl

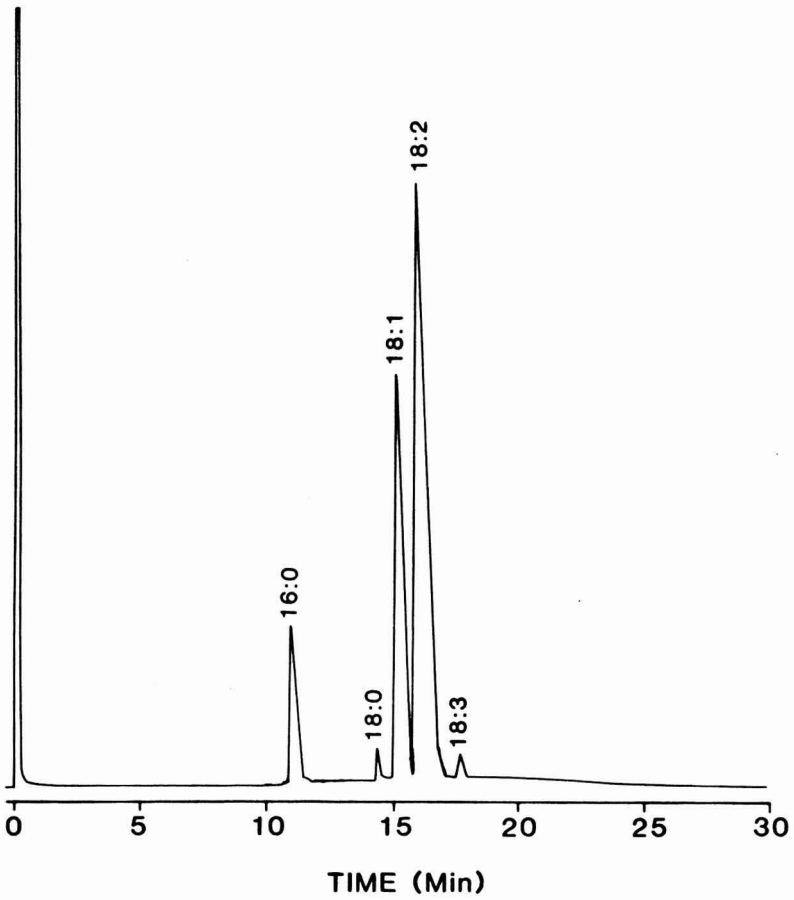

FIG. 2. GLC CHROMATOGRAM OF CORN OIL METHYL ESTERS PREPARED BY 1.0 M METHANOLIC NaOH CATALYZED TRANSESTERIFICATION
The GLC conditions are presented in the text.

esters. To obtain quantitative information, pure triglycerides (tripalmitin, triolein, trilinolein, and trilinolenin) were transesterified with 1.0 M methanolic NaOH as well as with conventional transesterification (Metcalfe *et al.* 1966), which involves saponification followed by esterification with BF_3-methanol. The results of replicate analyses are shown in Table 1 and indicate that there is virtually no difference in the fatty acid profile of the pure triglycerides transesterified by both procedures. Quantitative and qualitative analyses of FAME products of 1.0 M methanolic NaOH catalyzed transesterification were compared to FAME products obtained by conventional transesterification involving saponification followed by esterification (Metcalfe *et al.* 1966) and literature FAME products reported for soybean oil. If allowances are made for small differences in fatty acid composi-

tions of soybean oil due to cultivar, location and growing conditions, the results compare favorably. Table 2 illustrates no significant differences in fatty acid methyl ester profiles of soybean oil prepared with 1.0 M methanolic NaOH when compared to conventional transesterification (Metcalfe *et al.* 1966) and literature values.

TABLE 1.
FATTY ACID (AS WT. % METHYL ESTERS) COMPOSITION OF STANDARD TRIGLYCERIDE MIXTURE ± STANDARD DEVIATION*

Fatty acids	1.0 M Methanolic NaOH	Conventional[1]
16:0	25.4 ± 0.5	25.2 ± 0.8
18:1	24.8 ± 0.3	24.9 ± 0.4
18:2	24.7 ± 0.4	25.2 ± 0.6
18:3	24.9 ± 0.4	24.6 ± 0.9

*Ten samples.
[1]Determined by the method of Metcalfe *et al.* (1966)

TABLE 2.
COMPARISON OF SOYBEAN OIL FAME PROFILES

Fatty acids (as methyl esters)	Mean (%) ± Standard Deviation*		
	Methanolic NaOH	Conventional[1]	Literature Values[2]
14:0	0.1 ± 0.09	0.1 ± 0.09	0.1
16:0	11.0 ± 0.06	10.8 ± 0.10	10.3 - 11.2
18:0	2.9 ± 0.08	3.8 ± 0.06	3.8 - 4.0
18:1	25.6 ± 0.07	24.0 ± 0.08	22.8 - 24.0
18:2	53.9 ± 0.08	53.8 ± 0.06	51.0 - 53.9
18:3	6.7 ± 0.07	6.9 ± 0.07	6.8 - 6.9

*Ten samples.
[1]Determined by the method of Metcalfe *et al.* (1966).
[2]United States Department of Agriculture (1979) and Allen, R. R. (1977)

Preparation of methyl esters catalyzed by methanolic sodium hydroxide transesterification required no heating, extraction, solvent removal or washing prior to GLC analysis and required about 3–5 min (including clean-up time) compared to 12–20 min required by conventional transesterification. Conventional transesterification required heating, extraction and solvent removal prior to GLC analysis. However, longer clean-up time was required for large scale preparation of methyl esters compared to small scale preparation and depended on the amount of triglyceride transesterified.

The yield of vegetable oil FAME, volume of solvent (pentane or anhydrous diethyl ether) required to dissolve the vegetable oil and the amount of methanolic sodium hydroxide required to catalyze large scale preparation of methyl esters are presented in Table 3. Theoretical yields of methyl esters recovered after extraction from pentane or anhydrous diethyl ether were 97–99% based on the average molecular weights of the FAME determined from GLC analysis. Soybean oil FAME (assuming a molecular weight of 278.01 based on the fatty acid composition) gave the greatest yield when 1000 g of soybean oil was transesterified. Acceptable yields of FAME were obtained with 3–4 volumes of methanolic sodium hydroxide by weight of the vegetable oil. The high yield of FAME obtained with 1.0 M methanolic sodium hydroxide catalysis offers a potential for the use of the reagent for preparation of methyl esters intended for food applications such as in the preparation of sucrose polyesters (Olestra) and other carbohydrate polyesters. The low cost of sodium hydroxide may be an added advantage for wide use in large scale transesterifications. The 1.0 M methanolic sodium hydroxide transesterification reagent is easy to prepare compared to sodium metal in methanol or conventional transesterification reagent.

TABLE 3.
YIELDS OF METHYL ESTERS FROM VEGETABLE OILS TRANSESTERIFIED WITH 1.0 M METHANOLIC NaOH

Oil	Weight of oil (g)	Volume of solvent (ml)	Methanolic NaOH (v/w of oil)	Yield (wt.%)
Peanut	500	400	3.0	97
Corn	200	150	3.5	98
Soybean	1000	800	4.0	99
Safflower	100	100	3.0	97
Cottonseed	400	300	3.0	98

[1]Theoretical yields calculated from the average molecular weights of the FAME determined from GLC analysis

Solvents — Transesterification Media

Pentane and anhydrous diethyl ether were tested as solvents for vegetable oils instead of benzene, and transesterification was carried out with 1.0 M methanolic sodium hydroxide. Pentane and anhydrous diethyl ether were excellent solvents for 1.0 M methanolic sodium hydroxide catalyzed transesterification. For food applications, pentane and diethyl ether are recommended solvents for dissolving the vegetable oils and for methyl ester preparations with 1.0 M methanolic NaOH instead of benzene. Diethyl ether (Christie 1982) and pentane have the added advantage of being easy to evaporate. For routine analysis of methyl esters, evaporation of solvent is not required for accurate quantitation since concentration of FAME will change during evaporation and may result in inaccurate quantitation of the volatile fatty acid components of the oil. When 1.0 M methanolic sodium hydroxide was prepared and stored under anhydrous conditions, no change in transesterification catalytic capability was observed after 3, 7 or 30 days of storage at room temperature.

CONCLUSIONS

The 1.0 M anhydrous methanolic sodium hydroxide catalyzes rapid transesterification of vegetable oils. Transesterification catalyzed by methanolic sodium hydroxide eliminates the need for additional reagents such as methanolic HCl or BF_3 to stop saponification or "reverse" any saponification that may have occurred. Heating is not required for fatty acid methyl ester preparation. Methanolic NaOH is suitable for methyl ester preparations to be used in food, GLC analysis and other applications. Potentially hazardous solvents and reagents such as benzene are avoided when transesterification is catalyzed by methanolic sodium hydroxide. Pentane and anhydrous diethyl ether were excellent solvents for methanolic sodium hydroxide catalyzed transesterification of vegetable oils.

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NEW MODEL GEL SYSTEM FOR STUDYING WATER ACTIVITY OF FOODS

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ABSTRACT

A new model gel system consisting of agar and microcrystalline cellulose has been developed. The moisture equilibrium and diffusion properties have been measured. The D_{eff} for moisture is $10^{-9} m^2/s$, similar to cereals, and the system shows marked hysteresis with both swelling and structure collapse. The model system can be used over the whole range of water activity for studies on packaging moisture transfer and kinetics of chemical changes in diverse food systems.

INTRODUCTION

Food products are complex mixtures of solutes, water and insoluble solids. Numerous interactions, chemical reactions and structural changes take place during processing and storage. It is often difficult to study them directly in the actual food systems. Model food systems are simplified mixtures of food ingredients. Their composition and method of preparation are precisely known and controlled. Thus a particular physico-chemical aspect of the food product can be studied under known and precisely controlled but still realistic conditions.

Aqueous solutions have been widely used as model systems. Combinations of solutes and relative concentrations of them are infinite. However, it may be more accurate to incorporate a solid support or matrix in the model. Motoki *et al.* (1982) and Torres *et al.* (1985) used intermediate moisture food model systems based on a mixture of hydrogenated vegetable oil, sorbitol, isolated soy protein, caseinates, glycerol and water in varying amounts. The paste was then stuffed into a cellulose casing and adjusted to the desired water activity. Aqueous polysaccharide and gel matrices have been used extensively to study diffusion phenomenon

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(Busk and Labuza 1979; Furuta *et al.* 1984; Hendricks *et al.* 1986; Menting *et al.* 1970). Saravacos (1965) and Saravacos and Stinchfield (1965) studied the moisture sorption properties of model food gels (starch, gelatin, pectin, etc.). Guilbert *et al.* (1985), Giannakopoulos and Guilbert (1986) and Biquet and Guilbert (1986) used agar gels with various solutes and lipids, and different moisture contents to study sorbic acid and water diffusion. Roth and Loncin (1985) also used agar gels to study evaporation inhibitors. These agar gels showed ver interesting properties. Maloney *et al.* (1966) were the first to develop a model food system consiting of microcrystalline cellulose (MCC) blended with oil, water and various solutes. The ingredients were blended and freeze-dried prior to further adjustment of the water activity at the desired level. This systme was extensively used by the researchers at M.I.T. to study both lipid oxidation and nonenzymatic browning in foods. Hoskin and Dimick (1980) studied the physical property changes in chocolate during conching using scanning electron microscopy of model systems containing known amounts of cocoa solids, microcrystalline cellulose, starch, sucrose and lecithin.

The physico-chemical characteristics of agar have been extensively reviewed (Arnott *et al.* 1974; Busk 1978; Glicksman 1983; Igoe 1982; Izumi 1971; Meer 1977; Morris 1986) and MCC (Ganz 1977; Whistler and Daniel 1985). Moisture adsorption and desorption isotherms have been published for MCC (Bandyopadhyay *et al.* 1980; Iglesias *et al.* 1980; Johnston and Duckworth 1985; Spiess and Wolf 1983; Wolf *et al.* 1980) and agar (Duckworth 1972; Johnson and Duckworth 1985; Wallingford and Labuza 1983). The water activity subgroup of Cost to the E.E.C. Committees proposed MCC as the reference material for determinatio of water sorption isotherms (Spiess and Wolf 1984; Wolf *et al.* 1980). A "most probable" sorption isotherm for MCC was established. It was also used as the standard for an isopiestic a_w determination method (Vos and Labuza 1974).

Despite their versatility, the model systems developed at M.I.T., by Cost and by Guilbert and his collaborators, have some problems. The MCC model system is prepared by dry blending of the components. In some cases (depending on the formulation), it is difficult to obtain a homogeneous dispersion of the model system components, especially on a small scale. Mixing of these components in solution is also inadequate, since the MCC may precipitate out of solution and phase separation would occur when oil is present. The paste is also not very concenient to handle. On the other hand, agar gels have very good mechanical properties, and can be prepared in different geometries and formulations but are limited in their range of water activities. The lowest a_w reported was 0.64 and this was obtained with 70% w/w glycerol. Gelation requires a large amount of water and the gels shrink extensively on desorption. There is no reported work on attempts to freeze-dry agar gels. The agar gel network provides a convenient matrix but not the equivalent for a solid, inert support like MCC. Combination of both of them can

provide an interesting basis for an improved model food system. In this study the development and characterization of an agar/microcrystalline cellulose model system is presented.

MATERIALS AND METHODS

Model System Preparation

An agar/microcrystalline cellulose gel was developed with a constant MCC to agar ratio of 5/1.5 (w/w). About 1,050 g of the final gel suspension can be prepared conveniently at one time. Purified agar (15.75 g) (Difco Laboratories, Detroit, MI) was first dispersed into distilled wa5ter (981.75 g). The dispersion was then placed into a glass jar provided with a hermetic lid (aluminum foil plus screw cap lid) and was allowed to stand for about 16 h at 6°. It was next placed in a cold water bath. After heating to boiling, the gel suspension was kept for 20 to 35 min (depending on the quantity of gel prepared) in the boiling water. The suspension was then cooled down in a water bath at 43 °C until it reached this temperature. It was then immediately removed and added into a beaker containing the correct amount of MCC (52.5 g). The MCC used was Avicel PH 105 with an average particle size of 20 microns (F.M.C. Corporation, Philadelphia, PA). The mixture was immediately homogenized with a high speed mixer for 20 s (Moulinex Regal, Model No. V748). The suspension was quickly poured into plastic Petri dishes (60 × 15 mm), precooled at −27 °C. Approximately 30 to 32 g of mix was used per dish and the operation was performed as fast as possible in a freezer at 27 °C. The gels were removed after 6–7 min at this temperature and stabilized at 6 °C for 1 h. The final composition of the model system prior to drying was 1.5 g agar 5 g MCC and 93.5 g water/100 g gel.

After stabilization, 25 dishes of gels were placed into a metallic tray (42.5 × 29.5 × 4.5 cm) on wire netting with a small space (about 2 mm deep) between the botton of the tray and that of the idshes. The tray was then placed into a −27.5 °C freezer and about 3.7 liters of liquid nitrogen were continuously poured onto the tray. The geometry of the system and the quantity of liquid nitrogen are such that the latter overflowed the top of the gels. The gels were stabilized for 1 h at −27 °C and then freeze-dried for at least 96 h with a condenser temperature of −50 °C, and a pressure at the end of drying of less than 200 μ Hg in the freeze drier chamber (Virtis Research Equipment, Gardiner, NY). In this process, 5 to 15% of the gels were lost because of formation of cracks during the lyophilization process. In order to control the geometry and mass of the gel, they then can be trimmed with a razor blade. The average diameter of the gels was 4.1 cm and the average height was 1.1 cm. The average dry weight of a single sample was 1.1 g. The gels are then stored over desiccant (drierite) for at least 4 days before utilization.

Moisture Content Determination

Moisture contents were determined by the Karl Fischer method (Sholz 1984) using the Aquatest IV titrator (Photovolt Corp., New York) with hydronal-Coulomat reagent (Fisher Scientific Co., Fair Lawn, NJ). Extraction of the moisture was achieved by letting the samples stand in anhydrous methanol (ACS grade, Columbus Chemical Industries, Columbus, WI) for 12 h at 6°C.

Determination of Moisture Sorption Isotherms

Saturated salt solutions were prepared according to the procedure described by Multon (1984) and their water activity was taken from the literature (Greenspan 1977; Multon 1984; Stamp *et al.* 1984). Isotherms were determined using the method described by Labuza (1984) at 20 ± 1°C using triplicate samples. Equilibrium was believed to be reached when the change in weight expressed on a dry basis did not exceed 0.1%, i.e., ws less than 0.001 g/g dry solids for three consecutive weighings at no less than five days intervals. After equilibrium, each sample was immediately dropped into methanol for Karl Fischer titration.

Determination of the Diffusion Coefficients

The semi-continuous sorption system described by Lomauro (1984) was used with triplicate samples. The relative humidity in the head space above the sample is controlled by use of a specific saturated salt solution. The system was pre-equilibrated at the temperature of the experiment (20C) for 24 h prior to adding the samples. The gels were prepared and cut as indicated. They were initially weighed and then coated with silicone grease and aluminum foil on the side and botton to insure monodirectional sorption. After reweighing, they were then placed into the preweighed dish suspended in the system, at a temperature controlled to 20 ± 1°C. The weight was recorded as a function of time to 0.001 g (Mettler Instrument Corp., Princeton, NJ; Model PC 440). Initial and final thickness of the samples were measured to the nearest 0.02 mm with a modified Vernier (Draper Co.). The initial moisture content was determined by the Karl Fischer method on five samples prepared under the same conditions. The samples could be described geometrically as infinite slabs with moisture sorption taking place on one side only. For such a system and under certain assumptions, a simplified solution of Fick's second law can be used (Crank 1975; Schwartzberg 1975) and D_{eff} can be determined from a plot of ln Γ vs time as shown in Eq. (1).

$$\ln \frac{m - m_e}{m_o - m_e} = \ln \Gamma = \frac{\pi^2 \cdot D_{eff}}{L^2} \cdot t + \ln \frac{8}{\pi^2} \qquad (1)$$

where L is the average gel thickness, m, m_o and m_e are moisture contents (dry basis), respectively, at time t, zero, and at equilibrium.

RESULTS AND DISCUSSION

Times for equilibrium for gel samples placed at 85% relative humidity (initial gel a_w = 0. or 0.33) for adsorption or 33% relative humidity (initial gel a_w = 0.85) for desorption, at 20°C, ranged between 3 to 5 days depending on the experimental conditions. Spiess and Wolf (1984) reported equilibrium times of less than 4 days for MCC alone. The D_{eff} values are reported in Table 1. In comparison with the values of D_{eff} for many foods of 10^{-10} to 10^{-13} m^2/s (Biquet 1987; Hong *et al.* 1986), one can see that the values for these gels are higher, indicating a low internal resistance

TABLE 1.
EFFECTIVE DIFFUSION COEFFICIENT FOR FREEZE-DRIED M.C.C./AGAR GELS AT 20°C

Sorption Mode	Initial a_w	RH (%)	Thickness (mm)	$D_{eff} \cdot 10^9$ ($m^2 \cdot s^{-1}$)
Adsorption	0.33	85.1	10.51 (0.280)[a]	1.04 (0.102)[a]
Desorption	0.85	33.0	10.59 (0.300)	2.14 (0.069)

a (± standard deviation)

to the transport of moisture. The values fall in the range of thin dry crisp cereal products (Hong *et al.* 1986). It also appears that the D_{eff} values for desorption are double of that for adsorption. This could be due to a less dense gel network due to swelling on adsorption and/or to different transport mechanisms related to the higher moisture content. Figure 1 shows the adsorption and desorption isotherms at 20°C for the model gels. The desorption isotherm was determined after different pretreatments, i.e., pre-equilibration at 90.7, 85.1 and 75.4% relative humidity, respectively. A typical type II sigmoidal shape is seen of the Brunauer, Deming, Deming and Teller (B.D.D.T.) classification (also referred to as the B.E.T. classification — Gregg and Sing 1967). At water activities above 0.9, a collapse of the gel structure was usually observed. Hysteresis as seen in Fig. 1 is dependent on the adsorption history of the sample. Hysteresis mechanisms in porous solids such as food products are still not well understood. Mechanisms such as capillary condensation act only in rigid

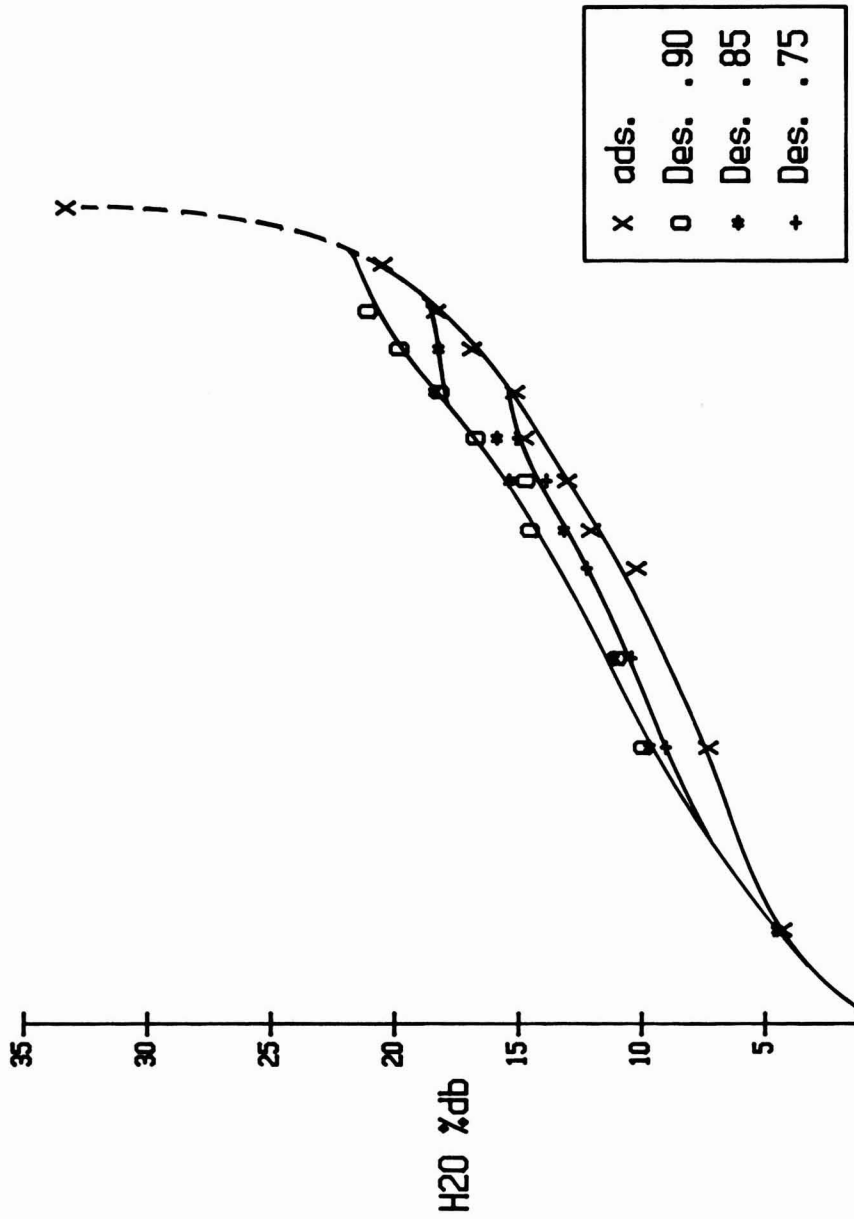

FIG. 1. ADSORPTION AND DESORPTION ISOTHERMS AT 20°C FOR THE MODEL GELS.

capillaries, not in swelling gel-like systems (Van den Berg 1986). The visible change of the gel structure at high water activities as well as the dependency of the desorption isotherm on the inital degree of adsorption seems to corroborate the hypothesis of an irreversibel change occurring in the pore structure upon adsorption.

The Guggenheim-Anderson-De Boer (G.A.B.) equation (Aguerre *et al.* 1986; Van den Gerg 1981;1983;1985) was evaluated for goodness of fit of moisture content versus water activity for the adsorption and desorption isotherms, using a direct non linear regression procedure. The three G.A.B. parameters are given in Table 2 (C and K are adsorption constants and m_o is the monolayer moisture content). These are typical for cereal foods as found by Lomauro *et al.* (1985a;b).

TABLE 2.
G.A.B. MOISTURE ISOTHERM FOR FREEZE-DRIED M.C.C. AGAR GEL AT 20°C

Food	Mode of Sorption	Initial a_w	Experimental a_w range	m_o $gH^2O/100$ g soluble	K	C
Freeze-dried M.C.C./Agar Gel	Adsorption	0.01	0.010–0.907	7.789	0.706	10.062
	Desorption	0.907	0.851–0.330	8.967	0.697	16.932
		0.851	0.808–0.112	10.648	0.603	9.416
		0.754	0.699–0.330	8.429	0.673	16.869

The freeze-dried MCC/agar gel system developed in this study appears to have a good potential for use as a food model system. The gel associates the advantages of a homogeneous porous solid matrix with a firm texture and an inert solid support as well as a typical type II moisture sorption isotherm representative of most food products. Different concentrations of reacting species can be obtained by dissolving them in the solution at the final step before freezing. The use of these gels is not limited to the dry and intermediate foods range since they may be used wet, i.e., without lyophilizatin for the higher water activities. In this case the water content added can be used to adjust the a_w, or solutes can be added for the same purpose. These gels are convenient to handle and their preparation is relatively simple. Potential uses range from packaging studies to mass transfer and deterioration kinetics experiments. Various solutes may be added and oil in water emulsions can be prepared within this gel matrix. Stamp (1987) used this model system to study the kinetics of aspartame degradation at different water activities. The preparation procedure was modified to prevent excessive loss of aspartame (a highly heat sensitive

compound) during the heating step. Heat sensitive solutes may be added to the gel suspension during the cooling process at a temperature depending on their thermal stability and the cooling of the system. In the case of a highly unstable compound such as aspartame, predissolution in cold water and addition to the agar/water gelling solution (at a temperature so that the final temperature after mixing was equal to 43 °C) just prior to adding the M.C.C. was also satisfactory provided that the quantity of added solution is small enough (1–3% w/w). The model gels were also used by Biquet and Labuza (1987) to evaluate the moisture barrier properties of a chocolate film in dry and intermediate moisture food model systems. Currently the non freeze dried gels are being used in our laboratory to study enzymatic browning and polyphenoloxidase activity as a function of water activity at high water activities.

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ACCEPTANCE AND STABILITY OF CHOCOLATE AND STRAWBERRY FOUNTAIN SYRUPS MADE WITH CONCENTRATED, DECOLORIZED, DEIONIZED HYDROLYZED WHEY PERMEATE[1]

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ABSTRACT

Two lots of whey permeate from ultrafiltration of sweet cheese whey were deionized, decolorized, treated with a β-galactosidase to hydrolyze lactose and concentrated. The second lot was heated to 75 C following concentration to decrease the potential for sugar crystallization.

Each lot of concentrated, decolorized, deionized hydrolyzed whey permeate (CHWP) was used in chocolate and strawberry fountain syrups as a partial or total sucrose replacement. Each syrup underwent organoleptic evaluation with comparison to a sucrose control. Samples of each syrup were also stored for 2 months at 4 C and 25 C.

Chocolate syrups made with complete replacement by CHWP lots 1 and 2 scored very high but only syrups made with CHWP lot 2 were stable after 2 months storage.

Strawberry syrups made with complete replacement by either lot of CHWP were sweet enough but scored poor in off-flavor intensity and overall preference. Syrups made with 50% replacement scored high in overall preference but poor in off-flavor intensity. Syrups made with greater than 50% replacement of sucrose became irreversibly crystallized and discolored after 2 months storage.

CHWP had acceptable flavor and sweetness for use in highly flavored fountain syrups and should be economical to produce and be competitive with fructose and corn sweeteners.

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INTRODUCTION

In the United States in 1983, an estimated 19.7 billion kg of cheese whey containing 179 million kg of protein were produced (Johnson *et al.* 1976; Kilara and Shahani 1979).

Sweet whey, which is about 5% lactose and 0.7% protein, has found little direct use in food applications. But fractionation of whey by ultrafiltration generates whey protein concentrate and whey permeate. Whey protein concentrate is used in dry mix foods but the permeate, which contains most of the lactose, is not used because of the low sweetness and solubility of lactose (Elmer and Clark, Jr. 1983; Kilara and Shahani 1979; Shah and Nickerson 1978).

Beta-galactosidase is used to enzymatically hydrolyze lactose to glucose and galactose. The resulting hydrolyzed whey permeate is sweeter and does not crystallize as readily (Shah and Nickerson 1978). Moreover, decolorizing is needed to remove interfering yellow-green color and deionizing to control the salty flavor.

Concentrated, decolorized, deionized hydrolyzed whey permeate (CHWP) has potential as a sweetener in frozen desserts, candies, canned fruits, jams and jellies, baked goods, fountain syrups, soft drinks and as a honey substitute (Tweedie *et al.* 1978).

This research was done to examine effects of partial and total replacement of sucrose by CHWP in chocolate and strawberry fountain syrups.

METHODS AND MATERIALS

Preparation of CHWP

The decolorization, deionization, hydrolysis and concentration steps were done by Wisconsin Dairies Cooperative, Baraboo, WI.

Sweet whey from cheese production was ultrafiltered to give whey protein concentrate and whey permeate. The whey permeate contained 5–6% total solids, 4.5% lactose and 0.5% ash. Decolorization of the permeate was by an activated charcoal system (Dorr-Oliver, Inc., Stamford, CT), which removed riboflavin, a small amount of ash and any remaining protein. These cause a whey-like off-flavor as well as an off-color. The resulting whey permeate contained 10% total solids.

Electrodialysis was used to remove the majority of the ash; removal was primarily the monovalent ions which have the greatest impact on flavor. Some divalent ions were removed as well. Deionization was 90% complete.

Before hydrolysis, the whey permeate was adjusted to pH 4.5 with sulfuric acid. The permeate was then passed through a Damrace APH reactor (Damrow Co., Div. of DEC Intl., Fond du Lac, WI) to hydrolyze lactose to glucose and

galactose. The enzyme, β-galactosidase from *Aspergillus oryzae*, was immobilized on an MPS^R microporous plastic sheet. Maintaining a 3 min residence time and an operating temperature of 30 to 40 C resulted in 90% lactose hydrolysis. Levels of less than 90% could be achieved by mixing 90% hydrolyzed CHWP with decolorized, deionizd unhydrolyzed whey permeate in the proper proportions. Following hydrolysis, pH was readjusted to 6.0-6.5 using potassium hydroxide. The permeate was then concentrated to 65% total solids by vacuum evaporation.

Lots 1 and 2 of CHWP were produced as shown in Fig. 1. Each lot had a final concentration of 65% total solids and contained 90% hydrolyzed lactose.

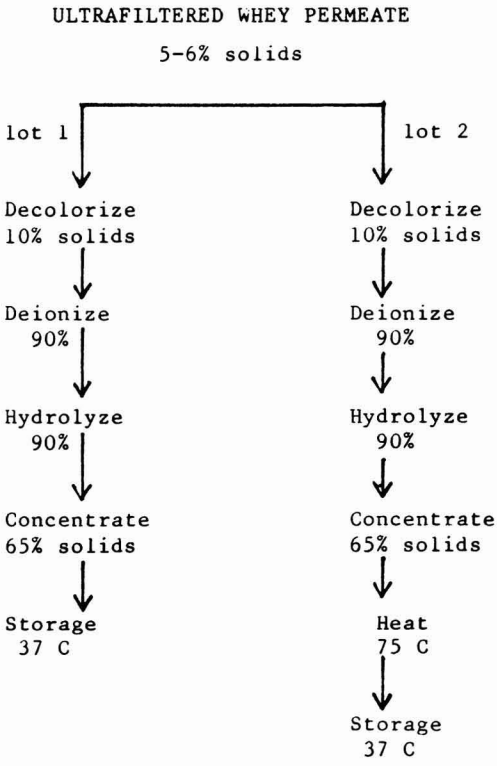

FIG. 1. STEPS IN PREPARATION OF TWO CHWP LOTS USED IN CHOCOLATE AND STRAWBERRY FOUNTAIN SYRUPS

Preparation of Fountain Syrups

Chocolate and strawberry fountain syrups were prepared with partial and total replacement of sucrose with CHWP. Pure sucrose and pure CHWP solutions of several different total solids levels were compared to determine a sweetness substitute ratio of CHWP for sucrose so that the original level of sucrose sweetness could be maintained.

All experimental and control sample batches were four liters. Samples were served over vanilla ice milk and evaluated by consumer taste panels.

Chocolate Fountain Syrup

Trial 1. One sample and one control were evaluated. Experimental sample 1 was prepared using the formula shown in Table 1. Complete replacement of sucrose with CHWP was possible because of favorable results with three preliminary batches made with gradually increasing levels of CHWP. The chocolate syrup control was made with sucrose. Both syrups were heated to 100 C and stored at 4 C.

Trial 2. Trial 2 duplicated trial 1 except that experimental sample 2 was made with CHWP lot 2.

Strawberry Fountain Syrup

Trial 1. One sample and two controls were evaluated. Experimental sample 1, strawberry syrup made with complete replacement of sucrose by CHWP lot 1, was prepared by the formula shown in Table 1. The lab-prepared control was

TABLE 1.
CHOCOLATE AND STRAWBERRY FOUNTAIN SYRUP FORMULAS

	Experimental sample		
	Chocolate	Strawberry	
Ingredient	trials 1 & 2	trials 1 & 2	trials 3 & 4
	%		
sucrose	---	---	23.11
CHWP solids	60.57	64.99	38.50
salt	0.04	---	---
vanilla	0.04	---	---
cocoa	8.56	---	---
strawberry extract	---	0.96	0.96
freeze dried beet powder	---	0.02	0.02
total solids	69.21	65.97	62.59

made with sucrose. After heating to 100 C, strawberry extract and freeze-dried beet powder were added for flavor and color, respectively.The commercial control was sweetened with sucrose and corn sweetener and all three samples were stored at 4 C.

Trial 2. Trial 1 was duplicated except that experimental sample 2 was made with CHWP lot 2.

Trial 3. Experimental sample 3 was prepared as in trials 1 and 2 but with 50% replacement of sucrose with CHWP lot 1. The formula is shown in Table 1. The same controls from trials 1 and 2 were used for comparison.

Trial 4. Trial 3 was duplicated except that experimental sample 4 was made with CHWP lot 2.

Storage Samples

A 100 mL sample of each experimental sample and control were stored at 4 C and 25 C. After 2 months, each sample was evaluated by the authors for appearance and stability of flavor and texture by comparison to freshly prepared syrup.

Descriptive Sensory Evaluation

Panelists for descriptive analysis of chocolate and strawberry syrups were experienced in the systematic evaluation of foods but not specificially trained in evaluating these products.

Panelists were seated in individual booths when evaluating products and were given approximately 80 mL of syrup over 120 g of vanilla ice milk. Each product variation was coded with a different 3-digit number. Prepared ballots listed selected attributes for evaluation on quantitative analysis scales. Following analysis, marked ballots were coded by computer with a program for analysis of variance appropriate for a randomized complete block design. Mean scores for sample attributes, F-values and least significant differences at a 5% level were computed for each set of samples when significant F-values were found.

RESULTS AND DISCUSSION

In all panel sensory evaluations, viscosity, sweetness intensity, off-flavor intensity and overall preference were the sample attributes of importance. Texture, appearance and chocolate or strawberry flavor were evaluated but results were not as strongly related to the amount of CHWP as those given previously.

Experimental samples evaluated in trial 1 with both syrups were prepared seven days after CHWP lot 1 was produced. At that time, sugar caramelization in the

CHWP was not visually apparent. However, experimental chocolate syrup evaluated in trial 2 and experimental strawberry syrup evaluated in trials 2, 3 and 4 were made 2 months after CHWP lot 2 production and caramelization in the CHWP was advanced. Caramelization made blending acceptable strawberry colors easier and did not affect the flavor of either syrup. In lighter colored or less intensely flavored syrups, the level of CHWP caramelization should be considered a drawback.

Chocolate Fountain Syrup

In trial 1, experimental sample 1 compared favorably with the control in every category. There were no significant differences between the two in sweetness intensity, off-flavor intensity of overall preference. The faint whey-like flavor of the CHWP was easily masked by the intense cocoa flavor. Sweetness intensities of the samples were similar even though CHWP imparted a different sweetness character than sucrose.

In trial 2 also, there were no significant differences between experimental sample 2 and the control in sweetness intensity, off-flavor intensity and overall preference. Use of CHWP lot 1 or lot 2 made no significant difference in the panel evaluation of chocolate fountain syrup. The statistical results from taste panel evaluations of trial 2 are shown in Table 2.

After 2 months storage at 4 C, experimental sample 1 became extremely viscous and crystalline and its acceptable flavor was masked by this textural defect. Vigorous stirring of the sample improved its viscosity. Crystallization, however, could only be reversed by reheating to 100 C. Experimental sample 2 did not crystallize, retained good flavor but separated and became slightly more viscous. These problems were corrected by shaking. The control sample remained smooth but viscosity increased with time. Samples stored at 25 C were the same as their counterparts stored at 4 C.

The increase in viscosity is expected in a commercial chocolate syrup. As long as shaking returns normal viscosity, this was not considered a defect. The phase separation could be minimized by adding starch.

If the syrup is to be served cold, any crystallization is objectionable. But if the syrup is heated before serving, crystallization will be eliminated and a syrup with complete replacement of sucrose by CHWP lot 1 would be acceptable. Syrups made with complete replacement with CHWP lot 2 would be acceptable hot or cold because the post-pasteurization heat treatment prevented crystallization.

Strawberry Foutain Syrup

In trial 1, both controls scored significantly better than experimental sample 1 in off-flavor intensity and overall preference. The strawberry flavor was not strong enough to cover the whey-like flavor of CHWP lot 1.

TABLE 2
SUMMARY OF MEAN SCORES FOR THE DESCRIPTIVE SENSORY ANALYSIS OF CHOCOLATE FOUNTAIN SYRUP, TRIAL 2 AND STRAWBERRY FOUNTAIN SYRUP, TRIAL 4

Product	Sample attributes: Sweetness intensity[1]	F	LSD	vis-cosity[2]	F	LSD	Off-flavor intensity[3]	F	LSD	Overall preference[4]	F	LSD[5]
	Mean scores											
Chocolate fountain syrup, trial 2[6]												
Experimental sample 2	4.31[a]			3.73[a]			2.83[a]			4.25[a]		
		NS	0.55		NS	0.47		NS	0.43		NS	0.47
Control chocolate syrup	4.14[a]			2.37[a]			2.92[a]			4.03[a]		
Strawberry fountain syrup, trial 4[7]												
Experimental sample 4	4.81[ab]			3.66[b]			3.90[b]			3.34[ab]		
Lab-prepared control	4.25[b]	S	0.58	2.78[a]	S	0.46	3.24[a]	S	0.74	2.93[b]	S	0.58
Commercial control	5.00[a]			2.96[a]			2.98[a]			3.59[a]		

[1]Scale: 1 = very weak; 7 = very strong.
[2]Scale: 1 = thin; 7 = thick.
[3]Scale: 1 = imperceptible; 7 = extremely pronounced.
[4]Scale: 1 = dislike extremely; 7 = like extremely.
[5]LSD at 5% level.
[6]n = 29.
[7]n = 24.
S = significant at 5% level; NS = not significant.
[a,b]Mean scores in same column with same superscript are not significantly different at 5% level.

In trial 2, both controls scored significantly better than experimental sample 2 with less off-flavor intensity. However, the commercial control was preferred over experimental sample 2 and the lab-prepared control. Experimental sample 2 was the most viscous of the three samples. The heating of CHWP lot 2 made no significant difference in the panel evaluation.

Trials 3 and 4 produced results that were nearly identical to each other, showing that the CHWP lot used made no significant difference in the flavor of the resulting syrup. The overall preference of the experimental samples was better than that of the commercial control. The off-flavor, however, was still more intense than in both controls. This suggests that the off-flavor is not as negative as in the completely replaced syrup but that it actually may be somewhat pleasing. Once again, the experimental samples were more viscous than the controls. The statistical results of trial 4 were considered typical for all strawberry syrups made with CHWP in place of 50% of the sucrose and results of panel evaluations are shown in Table 2.

After 2 months storage at 4 C, both experimental samples 1 and 2 were thick, lumpy and crystallized and had changed from clear, dark red to opaque pink. At 25 C, these results were similar although the samples were slightly more fluid. This breakdown was not reversed by heating to 100 C. Experimental samples 3 and 4 retained their dark red color, smooth, easily pourable body and flavor at 4 C and 25 C as did the lab-prepared and commercial controls.

Because of flavor and stability problems, strawberry syrups made with 100% replacement of sucrose with CHWP are not acceptable. Syrups with 50% replacement of sucrose with CHWP may be acceptable if the off-flavor is not judged to intense for the intended use.

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