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EFFECTS OF PROCESSING ON THE THIAMIN, RIBOFLAVIN, AND VITAMIN B-12 CONTENT OF FERMENTED WHOLE GRAIN CEREAL PRODUCTS

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ABSTRACT

The concentrations of thiamin, riboflavin, and vitamin B-12 in fermented grain products from white wheat, red wheat, corn, milo, and barley were assayed microbiologically. Vitamin B-12 was produced during the fermentation and was present at levels of 190–560 ng/100 g distillers' grain (dwb). The effects of five different drying methods and of pH prior to drying the fermented mash on the levels of these three vitamins were determined for distillers' dried grains produced from white wheat. Thiamin was most labile during liquefaction and to the drying process. Relatively large amounts of these three vitamins were lost if the soluble solids were not recovered in the finished product.

INTRODUCTION

Studies reporting the nutritional analyses of distillers' grain products and similar materials have concentrated on the dietary fiber content (Dong and Rasco 1987; San Buenaventura *et al.* 1987), amino acid composition (Ranhotra *et al.* 1982; Wu *et al.* 1984; Chung and Pomeranz 1985; Dong *et al.* 1987), and protein quality (Satterlee *et al.* 1976; Ranhotra *et al.* 1982; Bookwalter *et al.* 1984; Dong *et al.* 1987). Although it is known that whole grains contain significant levels of thiamin and riboflavin (Zapsalis and Beck 1985), very little information has been reported on either the vitamin content or the effects of processing on the levels of vitamins, such as thiamin and riboflavin, in distillers' grain products. Other investigators have reported the content of B vitamins in wheat and the

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variability in vitamin content among several types and cultivars of wheat from different locales (Davis *et al.* 1984; Keagy *et al.* 1980). The levels of certain B vitamins in brewers' condensed solubles (Sebree *et al.* 1983) and in fermented corn products have been reported (Ranhotra *et al.* 1982; Murdock and Fields 1984; Chung and Fields 1986).

The purposes of this study were to determine: (1) the levels of thiamin, riboflavin, and vitamin B-12 in distillers' dried grain products made from different feedstocks; and (2) the effects that specific manufacturing procedures have on the content of these three vitamins in distillers' grain materials made from soft white wheat.

MATERIALS AND METHODS

Preparation of Distillers' Grain Materials in the Laboratory

Distillers' grains were prepared according to the procedures described by Rasco *et al.* (1987a) and Rasco (1988) from the following grains: soft white winter wheat (Hill 81 cultivar), hard red wheat (a blended flour containing the cultivars Fremont, Pilot and Bannock), whole ground #1 yellow dent corn, red sorghum (a blend of different cultivars of milo), and barley (a blend of different cultivars). Pilot plant experiments utilized the Hill 81 Cultivar of soft white winter wheat as the feed stock grains.

Selection and Treatment of Process Samples

Process samples were obtained after liquefaction, saccharification, and during fermentation. In addition, portions of the sample obtained at the completion of the fermentation prior to drying were either neutralized, neutralized and filtered, filtered, or washed (Rasco 1988). The targeted final moisture content for the dried products were 10–12%. The process samples to be freeze dried were frozen at −40°C in a blast freezer and lyophilized (Freezemobile 6, Virtis Co., Gardiner, NY). Process samples to be drum dried were immediately drum dried (Model ALC-4 standard atmospheric double drum dryer, 6″ × 8″, Blaw-Knox Food and Chemical Equipment Div., Buffalo, NY) at a steam pressure of 276–302 kPa and a speed of approximately 3 rpm. The drums were approximately 0.2 mm apart. These experiments were conducted three times.

Drying Methods for Distillers Grains Produced in the Pilot Plant

Distillers' grain products were dried in the laboratory using a freeze dryer or drum dryer as described above. Pilot scale quantities of distillers' dried grains were dried using a custom built tunnel dryer (POS Pilot Plant Corp., Saskatoon, SK, Canada), steam tube dryer (Dedert Co., Olympia Fields, IL), or a microwave dryer (MIVAC, McDonnell Douglas, St. Louis, MO). Soluble solids were con-

centrated to approximately 25% solids with a custom built flash evaporator (POS Pilot Plant Corp.).

In the pilot plant experiments, concentrated solubles were prepared by centrifuging the mash at 5800 × g to separate the soluble and suspended solids from the rest of the fermented mash. After centrifugation, the distillers' grains were neutralized to approximately pH 7 and then dried as described below for microwave, steam tube, and tunnel drying to produce distillers' dried grains (DDG). The supernate was concentrated by flash evaporation to 25% solids, yielding concentrated solubles. These solubles had a pH of approximately 3.5 and were stored at −20°C until assayed.

The microwave dryer was operated at 1.2 kW microwave power, a pressure of 2.7 kPa, and a drying temperature of 40°C. The total drying time was 160 min in the microwave dryer to attain a moisture content of ≤10%. An inlet temperature of 95°C and an average air velocity of 6.5 m/s were used for tunnel drying. The average wet bulb temperature in the presence of product was 23°C, and in the absence of product, 17.6°C. The steam tube dryer was operated at a steam pressure of 5 kPa, a feed rate of 1.1 kg/min, and a condensate rate of 0.36 kg/min. The feed and exhaust temperatures were 60°C and 58°C, respectively. For DDG prepared by a combination of steam tube and tunnel drying, four parts of the partially dry product from the steam tube dryer were backmixed with 1 part the centrifuge cake and redried. When the product moisture content reached 15%, the steam tube dried material was transferred to the tunnel dryer and dried to approximately 10% moisture.

Vitamin Assay Procedure

Samples for assay (ca. 1.0000 g) were analyzed for thiamin, riboflavin, and cobalamin. Solid samples were finely ground. Each sample was assayed four to eleven times for each vitamin. Two to three aliquot levels from each sample extract were used with each assay. Duplicate tubes were assayed at each aliquot level. A new standard curve was run with each assay. Standard addition and recovery experiments were conducted for each of the vitamins tested in this study to determine whether interfering substances were present in the distillers' grain samples which would affect the reliability of the assay. Data were analyzed by analysis of variance and Tukey's multiple comparison test (Zar 1984).

Freeze dried microbes were purchased from the American Type Culture Collection (Rockville, MD). The microbes were rehydrated in sterile physiological saline (0.85% NaCl, w/v) and maintained in standard culture media (Difco 1986). The organisms (*Lactobacillus fermentum* 36 (ATCC 9338), *Lactobacillus casei* subspp. *rhamnosus* (ATCC 7469), and *Lactobacillus leichmanii* (ATCC 7830) respectively, were used for microbiological assay of thiamin, riboflavin, and vitamin B-12.

The procedure of Sarett and Cheldelin (1944) as modified by the Association

of Vitamin Chemists (1985) and Difco (1986) was employed for measuring thiamin. The extraction procedure for thiamin was described by the Association of Vitamin Chemists (1985). A centrifugation step was added to this procedure when filtration became difficult.

The riboflavin content of the whole grains and distillers' grains samples was determined according to the procedure of the AOAC (1984), the Association of Vitamin Chemists (1985), Snell and Strong (1939), and Difco (1986). The extraction procedure for riboflavin was that of the Association of Vitamin Chemists (1985). A centrifugation step was also added to this procedure when sample filtration became difficult.

For the vitamin B-12 assay, finely ground samples (ca. 1.0000 g) were blended with 30 mL of deionized, distilled water and 0.05 mL of freshly prepared 1% (w/v) NaCN (98% ACS, #2052202; Aldrich Chemical Co. Inc., Milwaukee, WI). The suspension was adjusted to pH 4.6–5.0 with 1 N HCl. The assay media contained 0.4 to 2.5 μg/mL NaCN, within the range prescribed by Voight and Eitenmiller (1984) and Chin (1984). Sodium cyanide converted the less stable chemical forms of vitamin B-12 in foods to the more stable cyanocobalamin form (Chin 1984). Cyanocobalamin standard (crystalline, approx. 99%, #V2876, Sigma Chemical Co., St. Louis, MO) was prepared according to Section 43.176 of the AOAC (1984). The assay procedures described by AOAC (1984) and Capp *et al.* (1949) were employed for measuring vitamin B-12 activity.

RESULTS

The concentrations of thiamin, riboflavin and vitamin B-12 in whole grains and drum dried DDGS made from white wheat, red wheat, corn, barley, and red sorghum (milo) on a dry weight basis (dwb) are given in Table 1. In general, there was little difference in the concentrations of either thiamin or riboflavin in the whole grains and the respective DDGS made from them (Table 1). Compared to the corresponding starting grains, no changes were observed in thiamin content in the DDGS products. Compared to the starting grain, white wheat and corn DDGS increased in riboflavin content. Analyses of the contents of thiamin and riboflavin among the five starting grains and among the five DDGS products indicated some consistent differences. White wheat and white wheat DDGS had the highest thiamin levels. The riboflavin content was lowest in milo and barley and also in the DDGS prepared from milo and barley.

As a result of fermentation, DDGS had measurable levels of vitamin B-12 (Table 1). In contrast, the levels of vitamin B-12 in the whole grains were very low. Concentrations of vitamin B-12 ranged from approximately 200 ng/100 g for barley DDGS to over 500 ng/100 g for white wheat DDGS. The increase in vitamin B-12 content in DDGS could be accounted for by the increase in the

TABLE 1.
THIAMIN, RIBOFLAVIN AND COBALAMIN LEVELS IN WHOLE GRAINS[1] DISTILLERS' DRIED GRAINS WITH SOLUBLES (DDGS)[2] (DRY WEIGHT BASIS)

Sample	Thiamin[3] ug/100g	Riboflavin[4] ug/100g $\bar{X} \pm$ SD (n)[6]	Cobalamin[5] ng/100g
Milo	60 ± 20 (18)^{a}	60 ± 60 (15)^{a}	< 0.5[7] (3)
Milo DDGS	60 ± 20 (18)^{A}	100 ± 60 (16)^{A}	280 ± 140 (13)*^{AB}
Barley	70 ± 20 (17)^{ab}	70 ± 60 (16)^{a}	< 0.5 (3)
Barley DDGS	80 ± 20 (18)^{A}	110 ± 90 (18)^{A}	200 ± 70 (16)*^{B}
Red Wheat	90 ± 30 (15)^{ab}	250 ± 120 (16)^{bc}	< 0.5 (3)
Red Wheat DDGS	100 ± 30 (16)^{A}	280 ± 130 (16)^{B}	400 ± 180 (16)*^{A}
Corn Meal	120 ± 60 (24)^{b}	290 ± 60 (13)^{b}	< 0.5 (3)
Corn DDGS	110 ± 60 (24)^{A}	420 ± 60 (13)*^{C}	320 ± 180 (20)*^{AB}
Soft White Winter Wheat	180 ± 90 (32)^{c}	170 ± 120 (26)^{c}	< 0.5 (3)
White Wheat DDGS	190 ± 130 (27)^{B}	240 ± 140 (28)*^{B}	560 ± 130 (27)*^{C}

[1]See text for a complete description of the grains.
[2]DDGS was drum dried.
[3]Thiamin was measured using *Lactobacillus fermentum* (Sarett and Cheldelin 1944; AVC 1985)
[4]Riboflavin was measured using *Lactobacillus casei* (Snell and Strong 1939; AOAC 1984)
[5]Cobalamin was measured using *Lactobacillus leichmannii* (Capps *et al.* 1949; AOAC 1984)
[6]Mean ± standard deviation. The total number of aliquots read is indicated in parentheses. Four to eleven samples of each grain were extracted then assayed at 2–3 readable aliquot levels. Duplicate tubes were assayed at each aliquot level, then averaged prior to statistical analysis. Cobalamin levels were assayed in one sample of each of the whole grains.
[7]Below the detection limit of the microbiological assay described in footnote 5.
* = Significantly different ($p \leq 0.05$) from the corresponding unfermented whole grain. Two sample t-test (Zar 1984).
abc, ABC = Means in the same column not sharing a common superscript are significantly different ($p \leq 0.05$) by analysis of variance and the Tukey test (Zar 1984).

yeast population. Corn, barley, red wheat DDGS, and milo DDGS had lower levels of vitamin B-12 than the DDGS from white wheat.

There were no significant differences in the levels of thiamin, riboflavin, or cobalamin among the steam tube/tunnel, tunnel, or microwave dried DDG products (Table 2). The levels of these three vitamins in the concentrated solubles (dwb) recovered after centrifugation of the pilot plant experiments were significantly higher than in the distillers' dried grain materials produced in either the pilot plant or laboratory experiments.

Additional experiments were conducted to determine the effects of different processing steps such as the pH of the material prior to drying, and the drying method on levels of thiamin, riboflavin, and cobalamin. The method of drying

TABLE 2.
THIAMIN, RIBOFLAVIN AND COBALAMIN LEVELS IN DISTILLERS' DRIED GRAINS FROM WHITE WHEAT DRIED BY THREE PILOT SCALE DRYING SYSTEMS AND CORRESPONDING SOLUBLES FRACTION (DRY WEIGHT BASIS)

Sample	Thiamin ug/100g $\bar{X} \pm$ SD (n)[2]	Riboflavin ug/100g $\bar{X} \pm$ SD (n)[2]	Cobalamin ng/100g $\bar{X} \pm$ SD (n)[2]
Distillers' Dried Grains			
Steam Tube/Tunnel Dried, pH 7.0	90[a] ± 30 (11)	310[a] ± 100 (10)	190[a] ± 50 (12)
Tunnel Dried, pH 7.0	100[a] ± 40 (10)	220[a] ± 80 (10)	270[a] ± 60 (12)
Microwave Dried, pH 7.0	110[a] ± 30 (10)	220[a] ± 50 (10)	230[a] ± 50 (11)
Concentrated Solubles, pH 3.5	260[b] ± 70 (12)	820[b] ± 770 (10)	790[b] ± 290 (17)

[1]Vitamin concentration measured using the microbiological assays described in Table 1.
[2]Mean ± standard deviation. The total number of aliquots read is indicated in parentheses (see Table 1).
ab = Means in the same column not sharing a common superscript are significantly different at $p < 0.05$; analysis of variance and the Tukey test (Zar 1984).

(freeze drying versus atmospheric drum drying) had little consistent effect on the levels of thiamin in DDGS, DDG, and WDG that was either acidic (pH = 3.5) or neutral (pH = 7.0) prior to drying (Table 3). The level of riboflavin in freeze dried and drum dried WDG was similar regardless of the pH at which the product was dried (Table 4). In the different DDGS, DDG and WDG materials, the levels of riboflavin and vitamin B-12 were not significantly affected by either the pH of the fermented mash prior to drying or the drying method used (lyophilization versus atmospheric drum drying) (Tables 4, 5).

The effects of removing the soluble solids on the levels of these vitamins can be analyzed by comparing values for DDGS, DDG, and WDG dried at the same pH by the same type of dryer. Removing a portion of the soluble solids from DDGS to produce DDG had little effect on the concentrations of the three vitamins (Tables 3–5). The freeze dried soluble solids had thiamin and vitamin B-12 levels which were similar to those for DDGS and DDG but higher levels of riboflavin. The concentration of thiamin, riboflavin, and vitamin B-12 in the freeze dried solubles was generally higher than in WDG. The pH of the solubles prior to concentration and drying did not appear to adversely affect vitamin stability. Twenty to twenty-five percent of the total thiamin, riboflavin, and cobalamin content of the fermented mash was recovered in the soluble fraction (Tables 3–5), based on the recovery of 38% of the solids in the soluble fraction and 62% of the solids in the insoluble fraction of the fermented mash (data not shown).

TABLE 3.
THIAMIN CONTENT IN PROCESS SAMPLES AND DISTILLERS' GRAIN PRODUCTS FROM SOFT WHITE WINTER WHEAT[1] (DRY WEIGHT BASIS)

Sample[2]	Experiment I	Experiment II	Experiment III
	ug/100g $\bar{X}$ + SD (n)[3]		
Process Samples			
Liquefaction	70 ± 30 (12)^{ab}	ND[4]	ND
Saccharification	80 ± 20 (12)^{ac}	ND	ND
Fermentation			
0 hr.	ND	130 ± 60 (11)^{bcd}	ND
12 hrs.	80 ± 30 (12)^{ac}	ND	ND
24 hrs.	ND	170 ± 80 (12)^{cd}	ND
36 hrs.	130 ± 50 (18)^{ac}	ND	ND
60 hrs.	ND	190 ± 70 (12)^{d}	ND
DDGS			
A-FD	130 ± 60 (18)^{a}	ND	70 ± 30 (12)^{ab}
A-DD	110 ± 50 (12)^{ac}	150 ± 50 (12)^{cd}	100 ± 20 (10)^{ab}
N-FD	80 ± 20 (12)^{ac}	180 ± 70 (12)^{cd}	ND
N-DD	60 ± 20 (9)^{ac}	130 ± 50 (12)^{bcd}	ND
DDG			
A-FD	100 ± 40 (18)^{ac}	150 ± 70 (12)^{cd}	70 ± 20 (12)^{ab}
A-DD	90 ± 40 (18)^{ac}	120 ± 40 (12)^{abc}	90 ± 40 (13)^{ab}
N-FD	100 ± 30 (12)^{ac}	130 ± 60 (12)^{bcd}	ND
N-DD	90 ± 20 (12)^{ac}	120 ± 140 (12)^{abc}	90 ± 30 (10)^{ab}
WDG			
A-FD	60 ± 30 (12)^{c}	70 ± 50 (12)^{ab}	110 ± 40 (11)^{a}
A-DD	90 ± 50 (12)^{ac}	70 ± 40 (12)^{ab}	70 ± 20 (12)^{b}
N-FD	ND	50 ± 20 (12)^{a}	ND
N-DD	ND	70 ± 20 (12)^{ab}	ND
Soluble Solids			
A-FD	100 ± 40 (18)^{ac}	120 ± 30 (18)^{abcd}	ND
N-FD	90 ± 30 (18)^{ac}	140 ± 30 (20)^{bcd}	ND

[1]See text for preparation of distillers' grain materials.
[2]DDGS = Distillers' dried grains with solubles
DDG = Distillers' dried grains
WDG = Washed distillers' grains
A = Acidified - pH 3.5
N = Neutralized - pH 7.0
FD = Freeze dried
DD = Drum dried
[3]Mean ± standard deviation. The total number of aliquots read is indicated in parentheses as described in Table 1.
[4]ND = Not Determined
a-d = Means in the same column not sharing a common superscript are significantly different at $p \leq 0.05$; analysis of variance and the Tukey test (Zar 1984).

TABLE 4.
RIBOFLAVIN CONTENT IN PROCESS SAMPLES AND DISTILLERS' GRAIN PRODUCTS FROM SOFT WHITE WINTER WHEAT[1] (DRY WEIGHT BASIS)

Sample[2]	Experiment I	Experiment II	Experiment III
	ug/100g $\bar{X}$ + SD (n)[3]		
Process Samples			
Liquefaction	140 ± 50 (11)[a]	ND	ND
Saccharification	160 ± 80 (10)[a]	ND	ND
Fermentation			
0 hr.	ND	270 ± 110 (12)[abc]	ND
12 hrs.	170 ± 70 (10)[ab]	ND	ND
24 hrs.	ND	330 ± 110 (12)[bcd]	ND
36 hrs.	410 ± 180 (17)[def]	ND	ND
60 hrs.	ND	420 ± 80 (12)[def]	ND
DDGS			
A-FD	450 ± 80 (18)[def]	ND	170 ± 100 (12)[a]
A-DD	500 ± 60 (12)[f]	410 ± 80 (12)[de]	280 ± 50 (9)[a]
N-FD	330 ± 200 (12)[bcde]	520 ± 130 (12)[f]	ND
N-DD	350 ± 200 (12)[cdef]	410 ± 60 (12)[de]	ND
DDG			
A-FD	450 ± 150 (18)[def]	380 ± 60 (12)[cde]	190 ± 120 (12)[a]
A-DD	440 ± 90 (18)[def]	390 ± 170 (12)[de]	170 ± 100 (16)[a]
N-FD	480 ± 90 (12)[ef]	460 ± 150 (12)[ef]	ND
N-DD	290 ± 80 (12)[abcd]	390 ± 60 (12)[de]	300 ± 60 (10)[a]
WDG			
A-FD	230 ± 100 (12)[abc]	200 ± 40 (10)[a]	140 ± 70 (10)[a]
A-DD	220 ± 60 (9)[abc]	280 ± 50 (10)[abc]	180 ± 110 (12)[a]
N-FD	ND	250 ± 40 (8)[ab]	ND
N-DD	ND	210 ± 60 (10)[a]	ND
Soluble Solids			
A-FD	420 ± 100 (18)[def]	380 ± 70 (18)[cde]	ND
N-FD	430 ± 100 (18)[def]	400 ± 50 (20)[de]	ND

[1]See text for preparation of distillers' grain materials.
[2]DDGS = Distillers' dried grains with solubles
DDG = Distillers' dried grains
WDG = Washed distillers' grains
A = Acidified - pH 3.5
N = Neutralized - pH 7.0
FD = Freeze dried
DD = Drum dried
[3]Mean ± standard deviation. The total number of aliquots read is indicated in parentheses as described in Table 1.
[4]ND = Not Determined
a-f = Means in the same column not sharing a common superscript are significantly different at $p \leq 0.05$; analysis of variance and the Tukey test (Zar 1984).

TABLE 5.
COBALAMIN CONTENT IN PROCESS SAMPLES AND DISTILLERS' GRAIN PRODUCTS FROM SOFT WHITE WINTER WHEAT[1] (DRY WEIGHT BASIS)

Sample[2]	Experiment I	Experiment II	Experiment III
	ng/100g $\bar{X}$ + SD (n)[3]		
Process Samples			
Liquefaction	40 ± 20 (4)[a]	ND	ND
Saccharification	40 ± 20 (4)[a]	ND	ND
Fermentation			
0 hr.	ND	60 ± 40 (5)[a]	ND
12 hrs.	200 ± 40 (12)[b]	ND	ND
24 hrs.	ND	150 ± 70 (12)[a]	ND
36 hrs.	350 ± 60 (12)[c]	ND	ND
60 hrs.	ND	580 ± 110 (12)[de]	ND
DDGS			
A-FD	510 ± 130 (12)[de]	ND	270 ± 90 (12)[a]
A-DD	550 ± 70 (12)[e]	480 ± 70 (12)[cd]	310 ± 70 (12)[a]
N-FD	550 ± 100 (11)[de]	550 ± 120 (12)[cde]	ND
N-DD	540 ± 50 (12)[de]	450 ± 90 (12)[c]	ND
DDG			
A-FD	450 ± 70 (12)[d]	470 ± 110 (12)[cd]	240 ± 60 (12)[a]
A-DD	500 ± 100 (12)[de]	550 ± 90 (12)[cde]	330 ± 100 (12)[a]
N-FD	520 ± 40 (11)[de]	530 ± 100 (12)[cde]	ND
N-DD	550 ± 60 (12)[e]	490 ± 130 (12)[cde]	280 ± 60 (12)[a]
WDG			
A-FD	330 ± 60 (12)[c]	280 ± 70 (12)[b]	250 ± 110 (11)[a]
A-DD	370 ± 70 (12)[c]	300 ± 60 (12)[b]	190 ± 120 (12)[a]
N-FD	ND	310 ± 40 (12)[b]	ND
N-DD	ND	320 ± 50 (12)[b]	ND
Soluble Solids			
A-FD	560 ± 70 (12)[e]	620 ± 70 (12)[e]	ND
N-FD	490 ± 60 (12)[de]	550 ± 70 (12)[cde]	ND

[1]See text for preparation of distillers' grain materials.
[2]DDGS = Distillers' dried grains with solubles
DDG = Distillers' dried grains
WDG = Washed distillers' grains
A = Acidified - pH 3.5
N = Neutralized - pH 7.0
FD = Freeze dried
DD = Drum dried
[3]Mean ± standard deviation. The total number of aliquots read is indicated in parentheses as described in Table 1.
[4]ND = Not Determined
a-e = Means in the same column not sharing a common superscript are significantly different at $p \leq 0.05$; analysis of variance and the Tukey test (Zar 1984).

The recovery of thiamin, riboflavin, and vitamin B-12 activity at various steps in the manufacturing process for distillers' grain products from soft white winter wheat is presented for three separate experiments in Tables 3–5. The variability in vitamin content among experiments (three separate pilot scale production runs) independent of assay variability was due to small differences in process variables (i.e., temperature and pH) and in the size of the yeast inoculum. Even with the experimental variation, there were certain trends that emerged from each experiment. The level of thiamin in the process samples was lower following liquefaction (Table 3) compared to the level in the whole ground soft white winter wheat (Table 1). Levels of riboflavin were similar before and after liquefaction, but analytical values were highly variable. No significant changes in the levels of the three vitamins from the liquefaction to the saccharification step were observed on a per g solids basis. During the 60 h yeast fermentation, there was no significant change in the level of thiamin (Table 3); however, there was a trend indicating an increase in riboflavin content after 12 h of fermentation compared to samples obtained immediately after liquefaction or saccharification (Table 4). The apparent increase in riboflavin content corresponded to a loss of solids in the fermented mash due to the conversion of glucose to carbon dioxide and ethanol and not to the production of vitamin during the fermentation (Wu *et al.* 1984, Rasco *et al.* 1987). The vitamin B-12 level increased approximately 10-fold from the end of saccharification to the completion of the fermentation (Table 5).

DISCUSSION

Removal of the soluble and suspended solids from the distillers' grain products had a greater effect on the level of thiamin, riboflavin, and cobalamin than either the pH or method of drying. In general, the levels of thiamin, riboflavin, and vitamin B-12 were highest for white wheat compared to the other starting grains; and for white wheat DDGS, compared to the other DDGS products. There was a significant increase in the riboflavin content in the corn and white wheat distillers' grain products on a dry weight basis and in the cobalamin content in all of the DDGS products relative to the levels in the unprocessed whole grains (Table 1). The thiamin and riboflavin content of wheat in this study was similar to that reported by Keagy *et al.* (1980) and Davis *et al.* (1984).

Up to 85% of the thiamin and 40–60% of the riboflavin were lost after fermentation and drying, based on the vitamin content of whole white wheat and the stoichiometric conversion of three grams of whole grain to one gram of distillers' grains with solubles (DDGS). Although measurable quantities of vitamin B-12 were produced during the fermentation, these levels were not high enough to qualify DDGS as a good source of the vitamin.

The loss of vitamin activity in the distillers' grain products dried at a neutral pH (pH 7.0) was not significantly different from similar materials dried at an acidic pH (pH 3.5). The lack of a pH effect on vitamin content was unexpected, since B vitamins are generally less stable at a higher pH (Zapsalis and Beck 1985). It is possible that the high buffering capacity of the fermented mash protected vitamins from thermal inactivation at the higher pH.

Drying systems similar to those used in commercial manufacture (i.e., steam tube/tunnel and tunnel drying) (Table 2) yielded levels of thiamin, riboflavin, and vitamin B-12 in DDG that were generally comparable to those for lyophilized DDG (Tables 3–5). It is possible that these vitamins were protected from the adverse effects associated with harsh drying methods by carbohydrate and protein in the suspended or insoluble solids fraction. Because large standard deviations were observed in the data, the data were reanalyzed by four different power transformations: log, square, square root, and reciprocal. Analysis of the data by plotting F value (obtained by analysis of variance) versus the power transformation revealed that no significant differences were obtained by transforming the data.

Substantial quantities of the three B vitamins, approximately 25% of the total vitamins in the fermented mash, were recovered in the soluble and suspended solids. Removal of the soluble and suspended solids by washing or solvent extraction has been used by investigators seeking to improve the sensory qualities of distiller's grain products from corn (Bookwalter *et al.* 1984, 1988). In our study, the deliberate removal of the soluble and suspended solids via a washing/rinsing step resulted in the largest decrease in thiamin and riboflavin of all the treatments examined. Washing involved four separate rinses of the insoluble residue with water. In the preparation of DDG, only the soluble or suspended solids which were easily removed by filtration or centrifugation at low gravitational force (5800 × g) were removed. Since the soluble and suspended solids were difficult to remove completely by filtration or centrifugation, it was not surprising that little difference in vitamin levels was observed between DDG and DDGS materials from the same experiment (Table 3-5). Recovering all or a major portion of the soluble and suspended solids (DDGS) was necessary to obtain the highest possible levels of thiamin, riboflavin, and vitamin B-12 in the finished product.

Vitamin B-12 produced by *Saccharomyces cerevisiae* during the fermentation of whole grain could contribute a small amount of vitamin B-12 to the diet. A 100 g serving of DDG or DDGS would contribute up to 10% of the U.S. RDA for this vitamin. The levels of vitamin B-12 produced during fermentation by *S. cerevisiae* were less than those reported for a mixed culture fermentation of corn meal by *Bacillus megaterium* (ATCC 13639) and *Enterobacter aerogenes*, where concentrations of up to 56 ng vitamin B-12/g were obtained (Chung and Fields 1986). Levels of riboflavin produced during the mixed culture fermentation

of whole corn were similar to those produced during the yeast fermentation of DDGS (4.5 mg/g) (Chung and Fields 1986).

Dried distillers' grains (DDG) or distillers' dried grains with solubles (DDGS) from wheat had levels of thiamin (100–200 mg/100 g) and riboflavin (200–300 mg/100 g) that were higher than the levels in unenriched wheat bread flour (80 mg thiamin/100 g; 60 mg riboflavin/100 g), lower in thiamin than enriched wheat bread flours (440 mg/100 g thiamin), and similar in riboflavin content of enriched wheat flour (260 mg/100 g riboflavin) (Pennington and Church 1985). Incorporation of DDGS into flour-based foods would enhance other important nutritional properties, primarily increased levels of dietary fiber and protein (Dong and Rasco 1987; Rasco *et al.* 1987a). DDGS have been successfully incorporated into bakery items and breading and batter mixes at 25–30% replacement levels for all-purpose flour (Rasco *et al.* 1987b; Rasco *et al.* 1987c). However, our study indicates that DDGS was a relatively poor source of thiamin and riboflavin because of losses during processing.

CONCLUSION

The type of drying techniques and the pH at which the product was dried did not appear to significantly affect the thiamin and riboflavin content in distillers' grain products. Measurable amounts of vitamin B-12 were produced during the yeast fermentation. Recovering the soluble and suspended solids was required for maximal levels of these B vitamins in distillers' grain products.

ACKNOWLEDGMENT

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EFFECT OF STORAGE CONDITIONS AND PACKAGING MATERIALS ON THE PHYSICOCHEMICAL, MICROBIOLOGICAL AND SENSORY PROPERTIES OF CORN DRY MASA FLOUR

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ABSTRACT

Dry masa (corn dough) flour was stored for 6 months in paper and polyethylene bags at ambient conditions (average of 36°C and 29% R.H.) and under different controlled conditions of temperature (25 and 40°C; 60% R.H.). The physicochemical, microbiological and sensory properties of the dry masa flours were evaluated throughout storage. Polyethylene bags had more resistance to tearing and a lower rate of water vapor transmission than paper bags. Therefore, dry masa flours stored in paper bags deteriorated faster than counterparts stored in polyethylene bags. For each packaging system, masa flours deteriorated at the lowest rate when stored at ambient conditions. Within the controlled conditions, dry masa flour stored in polyethylene bags at 25°C and 60% R.H. retained its properties for a longer time than counterparts stored at 40°C in polyethylene bags or flours stored in paper bags. The dry masa flour water absorption index and amylograph peak viscosity decreased as the level of deterioration increased. Fat acidity and sensory evaluations were closely related to changes in dry masa flour quality. The adsorption isotherm of the dry masa flour indicated that the corresponding moisture content for an Aw of 0.8 was 15.3%. The most common fungi found in the deteriorated flours were Aspergillus terrus, Aspergillus flavus *and* Aspergillus ornatus.

INTRODUCTION

Corn tortillas are the most important food in the diet of many people in Latin America. In the rural areas of Mexico, corn in the form of tortillas provides about 70% and 50% of the caloric and protein daily intake, respectively. Ac-

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cording to Paredes and Saharopulos (1983), the estimated per capita annual consumption of tortillas in Mexico is 120 kg. Tortillas are generally produced by following the ancient Aztec process in which the corn kernels are alkali-cooked with lime, steeped and stone-ground. The resulting masa is shaped into tortillas which are then baked on a hot-griddle or gas-fired oven (Rooney and Serna-Saldivar 1987). The use of dry masa flour is gaining popularity due to its prolonged shelf-life and convenience. In Mexico, commercial nixtamalized dry masa flours are used at home by about 20% of the population (Sanchez-Marroquin *et al.* 1987). Dry masa flour is manufactured by cooking corn with lime and then grinding the resulting nixtamal into masa. The wet masa is then dried, ground, sieved and blended to yield a shelf-stable product of desired color, particle size, pH, water uptake and flavor (Gomez *et al.* 1987). In Mexico, most of the commercial dry masa flour is packaged in 1 kg paper bags.

Little information is available about the effects of different packaging materials and storage conditions on the shelf-life of dry masa flours. Paredes and Mora (1983) reported that significant changes in fat acidity, sensory properties, protein digestibility and protein efficiency ratio occurred throughout storage of masa flour in dessicators containing saturated salt solutions to produce environments with relative humidities ranging from 55–83%.

The objective of this research work was to study the physicochemical, microbiological and sensory properties of dry masa flour packaged in two different types of materials (paper and polyethylene), stored at two controlled conditions (25 and 40°C; 60% R.H.) and at ambient temperature and relative humidity.

MATERIALS AND METHODS

Experimental

Commercial dry corn masa flour was obtained from MINSA mill of CONASUPO (National Company of Popular Subsistance) located in Los Mochis, Sinaloa, Mexico. The general procedure to produce the flour consists of cooking commercial white maize (75% amylopectin, 25% amylose) in 1.5–1.6% lime, pre-drying the resulting wet nixtamal (40–42% moisture) in ovens at a temperature of 288°C and grinding to produce a semidry masa. The masa is further dried in vertical towers to reduce its moisture to about 5–7%. The resulting product is ground into flour and screened into three particles sizes (US mesh 20, 40 and 60). The coarse and medium size flour particles are reground and incorporated into the bin which contains the fine particles.

A 70 kg dry masa flour lot was homogenized in a Hobart Mixer (Model AS200T). Then, 1 kg dry masa flour was placed in two different types of bags: (1) paper and (2) polyethylene. The 10 × 25 cm brown paper bags (produced from cellulose) are used commercially to package dry masa flour and had a wall

thickness of 5.02 mils. After filling, paper bags were sealed with glue in such a way that the headspace was practically nonexistent. The 25 × 25 cm transparent polyethylene bags had a thickness of 0.85 Mils and were heat sealed using a Sealobag machine (Hamilton Beach Model 402). As in the paper bags, the headspace within the package was practically nil. Duplicate bags of dry masa flours were stored for 6 months under the following conditions: (1) 25°C and 60% relative humidity (R.H.); (2) 40°C and 60% R.H. and (3) ambient temperature and relative humidity (an average of 36°C and 29% R.H. during the study). The six resulting experimental treatments were placed on metalic shelves inside sealed concrete silos of 36 cm diameter and 90 cm height. Packages were placed in such a way that they did not touch each other. Each silo was provided with a 15 watt light bulb which was controlled by an electric clock (Intermatic Master Control Model D-811) set at a 12 h interval. The bulbs were attached to the inside of the concrete disk that covered the silo. The temperature and relative humidity were controlled by an aeration system as shown in Fig. 1.

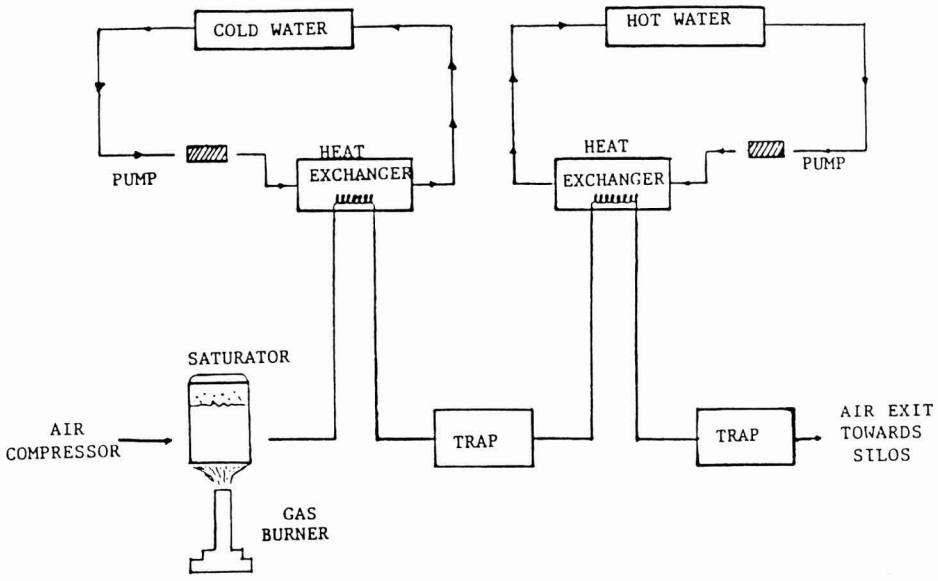

FIG. 1. DIAGRAM OF THE SYSTEM UTILIZED TO CONTROL TEMPERATURE AND RELATIVE HUMIDITY OF THE EXPERIMENTAL SILOS

The control treatment was stored at a temperature of 3–5°C in 5 kg transparent glass jars wrapped with aluminum foil. The purpose of the aluminum foil cover was to impede the penetration of light through the glass. Initially the airhead space was of approximately 10 cm, increasing after each sampling. At day zero

and after each sampling, the head space was flushed with nitrogen for 5 min before sealing the jar with its corresponding cap.

Physical Tests Used to Characterize Packaging Materials

The paper and polyethylene bags were characterized in terms of water vapor transmission (ASTM 1975), tearing resistance (ASTM 1979) and thickness. Film thickness was measured with an automatic micrometer (Model DDT, Type E.J., Cady & Co).

Adsorption Isotherm of Dry Masa Flour

The adsorption isotherm of dry masa flour was determined after equilibrating samples at different relative humidities in an air dessicator (Labuza 1984). The isotherm was determined at 25°C with salt solutions that provided different Aw's (0, 0.08, 0.22, 0.43, 0.57, 0.75, 0.84, 0.88, 0.97 and 1). Toluene was placed in those dessicators which contained solutions that provided relative humidities greater than 75% to inhibit mold growth. Samples were weighed every week to monitor gain or loss in moisture content. Samples reached equilibrium when its weight did not change more than 0.2 mg. The adsorption isotherm curve is the relationship between dry masa flour moisture content (Y axis) and water activity (X axis).

Analysis Used to Characterize Dry Masa Flours

Representative samples of dry masa flours were characterized according to standard AACC (1976) procedures for protein (method 46-13), moisture (method 44-40), fat (method 30-20), ash (method 08-03), crude fiber (method 32-15), pH (method 02-52) and fat acidity (method 02-03). Water absorption index was determined according to the procedures of Anderson *et al.* (1969) and Bedolla (1983). Amylograph peak viscosity of a suspension with 14% solids was determined using a Brabender Viscoamylograph (Type 800200).

Mold and Yeast Counts

Mold and yeast were quantitated at 0, 2, 4 and 6 months storage following AACC (1976) method 42–50. Eleven grams dry masa flour were mixed with 99 ml sterilized water and 10 g sterilized sea sand. Different dilutions were obtained and plated in acidic (pH 4–4.5) malt agar. Plates were incubated at 32°C for 72 h. Plates that contained less than 50 colonies were counted.

Fungi Identification

Molds of the genus *Aspergillus* were identified using the color and structural keys described by Raper and Fennel (1965). Molds were incubated at 25°C for

3–5 days in Czapeck-Dox agar and identified using a Stereoscope (Bausch&Lomb; Model ASZ 45L3) and a light microscope (Junior 2-Carl Zeiss, Model K-4-D).

Sensory Evaluation

Dry Masa Flour. A trained 8 member panel evaluated the color, odor, flavor and overall acceptability of the dry masa flours every month during the 6 month storage period. The panelists were trained before the beginning of the experiment and consisted of people who was familiar with the product and its characteristics. The test method was classified as affective and used a hedonic scale of 1 to 9 (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely). Panelists were offered all samples at the same time in an open area without any special lighting. Panelists were asked to take a drink of water before evaluations.

Tortilla. Tortillas were produced from dry masa flours stored for 6 months and then sensory evaluated by 30 untrained panelists. The dry masa flour was hydrated with water using a 1:1 ratio. The resulting dough was hand kneaded for 3 min and then subdivided into 40 g dough balls. Dough balls were molded into flat disks (2 mm thick) using a hand operated tortilla press. Tortilla disks were baked on a hot griddle at 300°C for 1.25 min (45 s on one side and 30 s on the other side). Tortillas were evaluated within the next 2 days for color, odor, texture, flavor and overall acceptability. A hedonic scale of 1 to 9 was used to evaluate the product (1 = dislike extremely; 5 = neither like nor dislike; 9 = like extremely).

Statistical Analysis

Sensory evaluations of dry masa flour, microorganisms counts, and changes in physicochemical properties of dry masa flour were analyzed as a factorial in a complete randomized experimental design in which factor A was the type of packaging system and factor B time of storage. When the interaction A × B was significant ($P < 0.05$), mean comparisons of the different packaging systems at each storage time were done using Least Significant Differences (LSD).

Data of sensory evaluation of tortillas was analyzed performing an analysis of variance (complete randomized design) test using the statistical analysis system (SAS 1979). Means were compared using Duncan's tests at a level of significance of 0.05.

RESULTS AND DISCUSSION

Physical Tests on Packaging Materials

Paper film was thicker (5.02 mils) than polyethylene film (0.85 mils). Despite the difference, the polyethylene film had six times as much resistance to tearing

as the paper film (171.9 g force vs 28.4 g force). The most noteworthy difference between the two types of materials was in their water vapor transmission rate. The polyethylene film had a very low rate of water vapor transmission (1.7 g water/day/m^2) when compared with paper film (774.6 g water/day/m^2). The rectangular configuration of the paper bag was kept throughout storage. On the other hand, the polyethylene bags did not have any particular configuration once they were filled with the masa flour. In addition, the transparent polyethylene film allowed the light to pass through the bag.

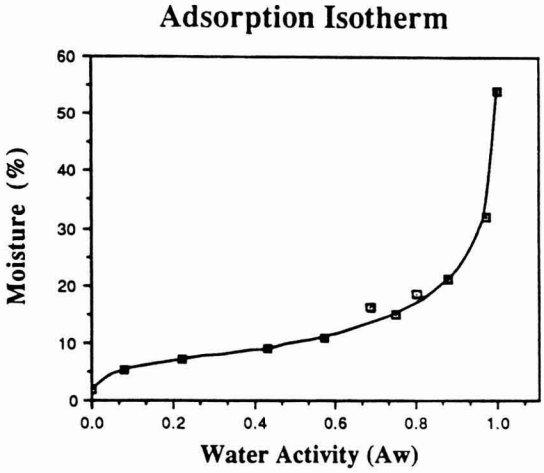

FIG. 2. ADSORPTION ISOTHERM OF DRY MASA FLOUR STORED AT 25°C

Adsorption Isotherm

Figure 2 shows the adsorption isotherm of dry masa flour stored at 25°C. Dry masa flour is generally packaged at a moisture content of less than 10%. At this moisture content the masa flour has a relative low Aw (0.5) in which microorganisms do not grow (Labuza 1984). There was a rapid increase in masa flour moisture content after reaching a water activity of 0.8, Aw in which most molds are able to grow (Labuza 1984; Labuza and Contreras-Medellin 1981). In other words, the dry masa flour was susceptible to microbial deterioration when it contained at least 15.3% moisture.

Changes in Chemical Composition

Table 1 shows the proximate analysis of the dry masa flours at day 0 and after 6 months at the different storage conditions and with the different packaging materials. Dry masa flours stored in paper bags had the highest moisture gain

throughout storage. The paper material had a very high rate of water vapor transmission so the masa flour was able to pick up water from the environment. The flour stored in paper bags at ambient conditions gained less moisture than the ones stored in the controlled conditions. This is due to the low average ambient relative humidity (29%) recorded during the experiment.

Flour stored in paper bags had the highest fat losses during storage. A close relationship between moisture gain and carbohydrate and fat loss was observed. Mold counts were very high in products packaged in paper bags and stored under a 60% controlled R.H. Mold enzymes might have broken down lipids and carbohydrates to simpler compounds or carbon dioxide, thus, decreasing the amounts of these nutrients. The partial loss of fat and nitrogen free extract components slightly concentrated the nitrogen fraction in the products stored in paper bags.

TABLE 1.
PROXIMATE ANALYSIS OF DRY MASA FLOUR PACKAGED IN PAPER AND PLASTIC BAGS AND STORED FOR 6 MONTHS UNDER DIFFERENT CONDITIONS OF TEMPERATURE AND RELATIVE HUMIDITY[a]

Storage Condition	Moisture	Ether Extract	Protein[b]	Ash	Crude Fiber	NFE[c]
	%					
0 Days Storage	7.48	5.18	10.15	1.69	1.40	74.55
6 Months Storage						
Control[d]	6.30	4.49	10.47	1.73	1.60	75.40
25°C; 60% Relative Humidity						
Paper	15.55	2.14	10.81	1.70	1.40	68.40
Plastic	6.80	4.36	10.38	1.70	1.22	75.54
40°C; 60 Relative Humidity						
Paper	12.22	3.68	10.48	1.80	1.18	70.80
Plastic	7.37	4.43	10.45	1.70	1.17	74.90
Ambient Condition[e]						
Paper	7.13	4.23	10.35	1.71	1.23	75.32
Plastic	6.70	4.49	10.11	1.67	1.25	75.76

[a]All values are expressed on dry matter basis. Means of six observations distributed in 2 silos.
[b]% Nitrogen × 6.25.
[c]Nitrogen free extract.
[d]Masa flour was stored in glass jars, flushed with nitrogen, covered with aluminum foil and refrigerated at 5°C.
[e]Average temperature of 36°C and 29% relative humidity.

Dry masa flours stored for up to 6 months in polyethylene bags contained similar moisture contents as the flour at day 0. The low film permeability decreased or impeded the diffusion of environmental moisture through the polyethylene material. Therefore, these flours maintained the moisture content and/or Aw at which microorganisms are inactive.

Figure 3 shows more detailed information about the moisture changes mentioned before. The effects of packaging materials and storage conditions on fat

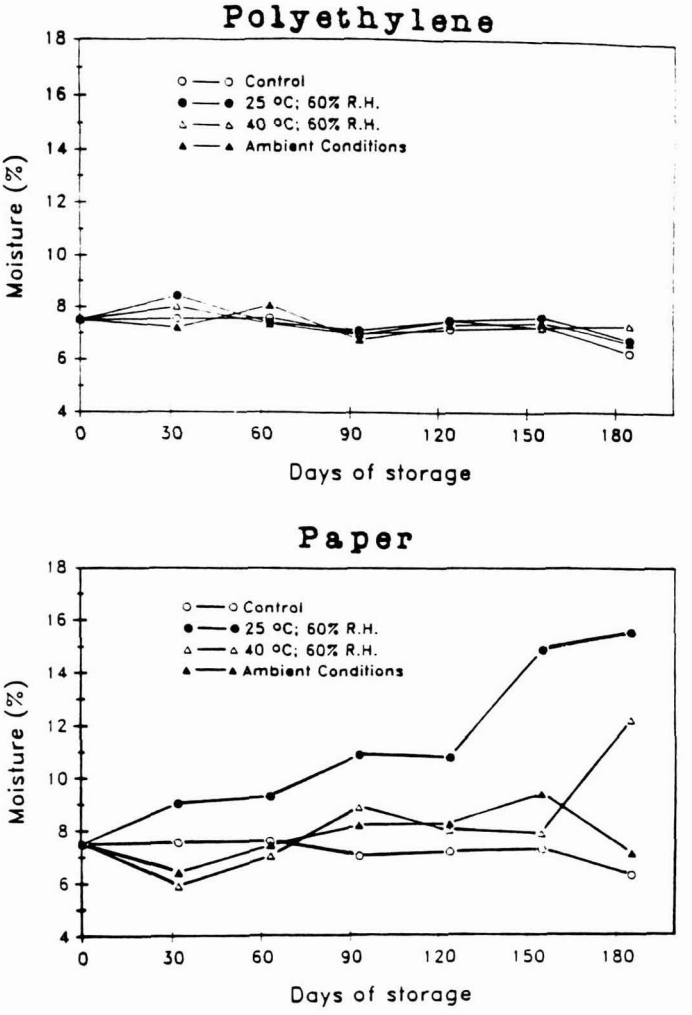

FIG. 3. EFFECT OF PACKAGING MATERIALS ON MOISTURE CONTENT OF DRY MASA FLOUR STORED FOR 6 MONTHS AT DIFFERENT TEMPERATURES AND AT AMBIENT CONDITIONS

acidity and flour pH are presented in Fig. 4 and Fig. 5, respectively. For all these variables, a significant interaction ($P < 0.01$) between time of storage and type of packaging system was found. Dry masa flour packaged in polyethylene bags contained a similar or even lower moisture than the original dry masa flour. The moisture content was always below 9%, thus, it was in the range that is

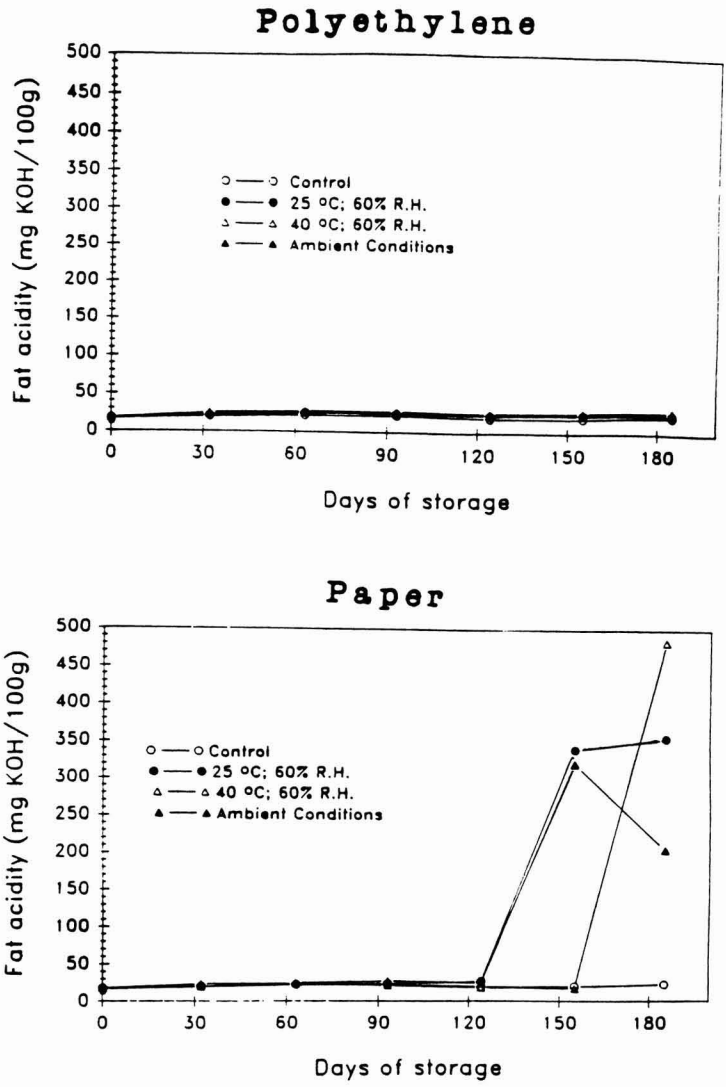

FIG. 4. EFFECT OF PACKAGING MATERIALS ON FAT ACIDITY OF DRY MASA FLOUR STORED FOR 6 MONTHS AT DIFFERENT TEMPERATURES AND AT AMBIENT CONDITIONS

difficult for the microorganism to grow (Fig. 2). On the contrary, flours packaged in paper bags and stored under controlled conditions gained moisture to a level that molds were able to grow and be active (Fig. 2, 3) (Labuza 1984; Labuza and Contreras-Medellin 1981). The rate of moisture gain was low during the first four months of storage. Afterwards, the flour stored at 25°C and 60% R.H. gained about 5% moisture (10.5–15.5%) in a two month period. A similar effect was observed during the last month of storage of the dry masa flour stored at 40°C and 60% R.H. Dry masa flour packaged in paper bags and stored at ambient conditions kept its original moisture content throughout storage. This is due to the high average temperature (39°C) and low relative humidity (29% R.H.) of the desert zone in which Hermosillo, Sonora, Mexico is located.

The fat acidity test, which gives an indication of the hydrolytic changes in lipids, steadily increased in dry masa flour packaged in paper bags and stored under controlled conditions. A dramatic increase in acidity values was observed after 4 months storage. At this particular point in time, the flour had the highest rate of moisture gain and the largest mold counts. These microorganisms broke down triglycerides into free fatty acids, thus, increasing acidity values. Similar results were obtained by Paredes and Mora (1983) when they stored limed maize meal under different relative humidities. Dry masa flour packaged in polyethylene bags contained similar fat acidity values throughout the six months of storage (Fig. 4).

Dry masa flour water absorption index decreased throughout storage in products stored in paper bags (Table 2). The water absorption index was significantly decreased due to the reduction in the starch or NFE fraction. The reduction was more pronounced in the flour stored at 40°C. The high temperature and relative humidity might have accelerated the process of starch breakdown by microorganisms, therefore, a significant change in the ability of the starch to retain the water was observed. Dry masa flours packaged in plastic materials did not change their water absorption index throughout storage, therefore, a higher tortilla yield could be expected in tortillas stored in polyethylene bags. A lower tortilla yield can be expected in flours stored in paper bags due to their lower water absorption index (Bedolla and Rooney 1982).

The changes in water absorption index were related to amylograph peak viscosity values. The control treatment has a slightly lower viscosity throughout storage time. The paste viscosity of dry masa flour packaged in paper bags decreased more throughout storage than their counterparts packaged in polyethylene bags. These changes in viscosity were due to amylolytic fungal activity that caused breakdown of starch.

Generally, dry masa flours have a pH ranging from 7–8.5. The pH is the result of the amount of lime used during cooking and the amount removed during nixtamal washing (Rooney and Serna-Saldivar 1987). The initial pH of the dry

TABLE 2.
WATER ABSORPTION INDEX AND PEAK VISCOSITY OF DRY MASA FLOUR PACKAGED IN PLASTIC AND PAPER BAGS AND STORED FOR 6 MONTHS UNDER DIFFERENT CONDITIONS OF TEMPERATURE AND RELATIVE HUMIDITY

	Water Absorption Index (g gel/g dry flour)			Amylograph Peak Viscosity[a] (Brabender Units)		
Months Storage	0	3	6	0	3	6
Storage Condition						
Control[b]	3.42	3.34	3.37	1609	1685	1410
25°C; 60% Relative Humidity						
Paper	3.42	3.29	3.33	1690	1675	1142
Plastic	3.42	3.37	3.45	1690	1585	1462
40°C; 60% Relative Humidity						
Paper	3.42	3.35	3.06	1690	1552	1207
Plastic	3.42	3.44	3.41	1690	1530	1412
Ambient Condition[c]						
Paper	3.42	3.25	3.27	1690	1485	1145
Plastic	3.42	3.27	3.37	1690	1567	1497
LSD[d]	N.S.	0.06	0.07	N.D.	N.D.	N.D.

[a]Peak viscosity of a slurry containing 14% solids.
[b]Masa flour was stored in glass jars, flushed with nitrogen, covered with aluminum foil and refrigerated at 5°C.
[c]Average temperature of 36°C and 29% relative humidity.
[d]LSD = Least significant difference ($P < 0.05$); NS = nonsignificant; N.D. = not determined.

masa flour was 7.5. Dry masa flour packaged in plastic bags showed slight changes in pH while flours packaged in paper bags maintained their pH up to 4 months storage. Afterwards, the products stored under controlled relative humidity had a sharp decline in pH (Fig. 5). This is the result of the high fungal growth observed during the last 2 months of storage. The metabolic activity of the fungi might have caused breakdown of nutrients (starch, protein, fats) into simpler compounds like organic acids, free fatty acids, etc. These acidic compounds lower the dry masa flour pH. The typical tortilla flavor was lost in these flours due to the lack of alkaline flavor and the presence of off-flavored compounds, likely produced by molds.

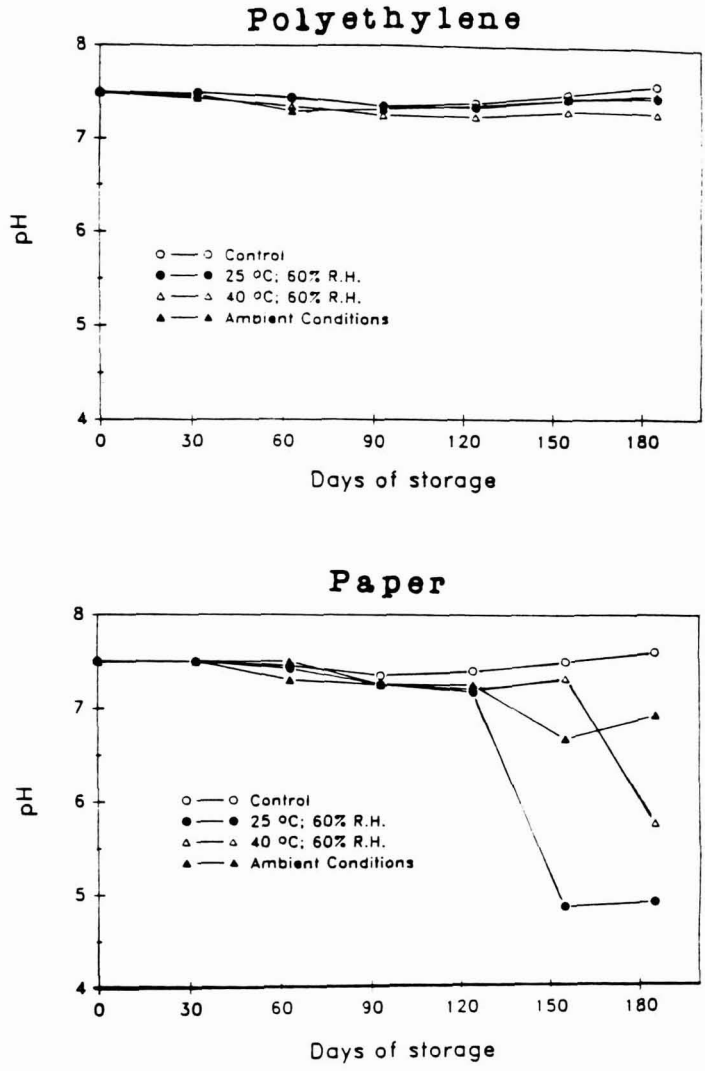

FIG. 5. EFFECT OF PACKAGING MATERIALS ON pH OF DRY MASA FLOUR STORED FOR 6 MONTHS AT DIFFERENT TEMPERATURES AND AT AMBIENT CONDITIONS

Microbiology

Table 3 shows the mold counts of the different dry masa flours throughout storage. Most of the microorganisms found in the different plates were molds. Yeasts were only found in very small quantities in flour packaged in paper bags and stored at ambient conditions and in the flour stored for 120 days at 40°C in

TABLE 3.
MOLD AND YEAST COUNTS (ORGANISMS/g) OF DRY MASA FLOURS PACKAGED IN PAPER AND PLASTIC BAGS AND STORED FOR 6 MONTHS UNDER CONTROLLED CONDITIONS OF TEMPERATURE AND RELATIVE HUMIDITY[a]

Storage Condition	Months Storage			
	0	2	4	6
Control	2.4×10^4	3.4×10^4	3.4×10^4	3.5×10^4
25°C; 60% Relative Humidity				
Paper	2.4×10^4	4.3×10^4	2.9×10^4	5.8×10^5
Plastic	2.4×10^4	4.3×10^4	1.1×10^2	1.4×10^2
40°C; 60% Relative Humidity				
Paper	2.4×10^4	5.6×10^4	3.2×10^4	1.0×10^5
Plastic	2.4×10^4	3.6×10^4	3.8×10^4	3.5×10^4
Ambient Condition[c]				
Paper	2.4×10^4	4.9×10^4	3.8×10^4	2.9×10^4
Plastic	2.4×10^4	5.4×10^4	3.1×10^4	3.4×10^4
LSD[d]	NS	1.0×10^4	0.6×10^4	1.7×10^4

[a]Means are averages of six replicas distributed in two silos.
[b]Masa flour was stored in glass jars, flushed with nitrogen, covered with aluminum foil and refrigerated at 5°C.
[c]Average temperature of 36°C and 29% relative humidity.
[d]LSD = Least significant difference ($P < 0.05$); NS = nonsignificant.

polyethlene bags. According to Labuza (1984) most molds are able to grow at Aw higher than 0.8. Flours packaged in paper bags and stored under controlled conditions contained similar mold counts up to 4 months storage. Mold counts dramatically increased during the fifth and sixth months of storage. As was expected, mold growth was closely related with the dramatic increment in flour moisture content and/or Aw (Fig. 2 and 3). Bothast *et al.* (1981) also found high mold counts in corn meal stored at 15 and 18% moisture after 3 months storage. The flour packaged in paper bags and stored under ambient conditions had low counts throughout the six months. The same was observed for all treatments packaged in polyethylene bags.

Most of the fungal contamination came from molds of the genus *Aspergillus*. The three most popular species that contaminated the flours were: *Aspergillus terrus, Aspergillus flavus* and *Aspergillus ornatus*. After 6 months storage, the

most common microorganisms that contaminated the flours packaged in paper bags and stored under controlled conditions were: *Aspergillus flavus, Aspergillus glaucus* and *Aspergillus ocraceus*. Bothast *et al.* (1981) reported *Aspergillus glaucus* and *Aspergillus candidus* as the predominant molds in corn meal stored for 6 months. Flours stored under ambient conditions in either paper or plastic bags contained *Aspergillus fumigatus* which is known to produce a respiratory disease in humans (Christensen and Kaufmann 1974).

Sensory Evaluation

Dry Masa Flour. Sensory evaluation of dry masa flours was closely related to the observed changes in physicochemical and microbiological properties previously discussed. Statistical analysis indicated that a significant interaction ($P < 0.05$) between storage time and type of packaging was found, therefore, differences between packaging systems at fixed storage times were determined. No significant differences ($P > 0.05$) among treatments during the first three months of storage were detected by panelists. A significant reduction ($P < 0.05$) in all sensory attributes was observed in products stored in paper bags during the last two months of the study. Interestingly, the most noticable change in chemical properties and microbiological counts occurred in flours stored under controlled conditions in paper bags during the last two months of storage. The most dramatic changes in color scores occurred in flours stored in paper bags under controlled conditions after 4 months storage (Fig. 6–7). Color changes might have been the result of pigment oxidation. Flours acquired a white-grayish color instead of the typical white-yellowish color (Fig. 6).

Panelists reported a moldy aroma in flours packaged in paper bags and stored for more than 5 months (Fig. 6). The moldy odor is the result of the high microbial counts found in these treatments (Table 3). The flours packaged in paper bags and stored under ambient conditions had a rancid odor after 155 days of storage. In addition, a visible insect growth was observed.

Flours packaged in polyethylene bags also showed a reduction in odor scores over time. The rate of reduction was smaller than the one found for treatments in paper bags (Fig. 6). Panelists detected a slight rancid aroma in products packaged in polyethylene bags (40°C; 60% R.H.) after 4 months storage.

Masa flours stored in paper bags under controlled conditions showed a dramatic reduction in flavor and overall acceptability ($P < 0.05$) after 4 months storage (Fig. 7). Panelists reported an astringent-sour taste as the most common off-flavor. These flours were not further tasted on the fifth and sixth month due to visible presence of molds. The pH change and high acidity values reported before (Fig. 4 and 5) were closely related with the reported changes in flavor.

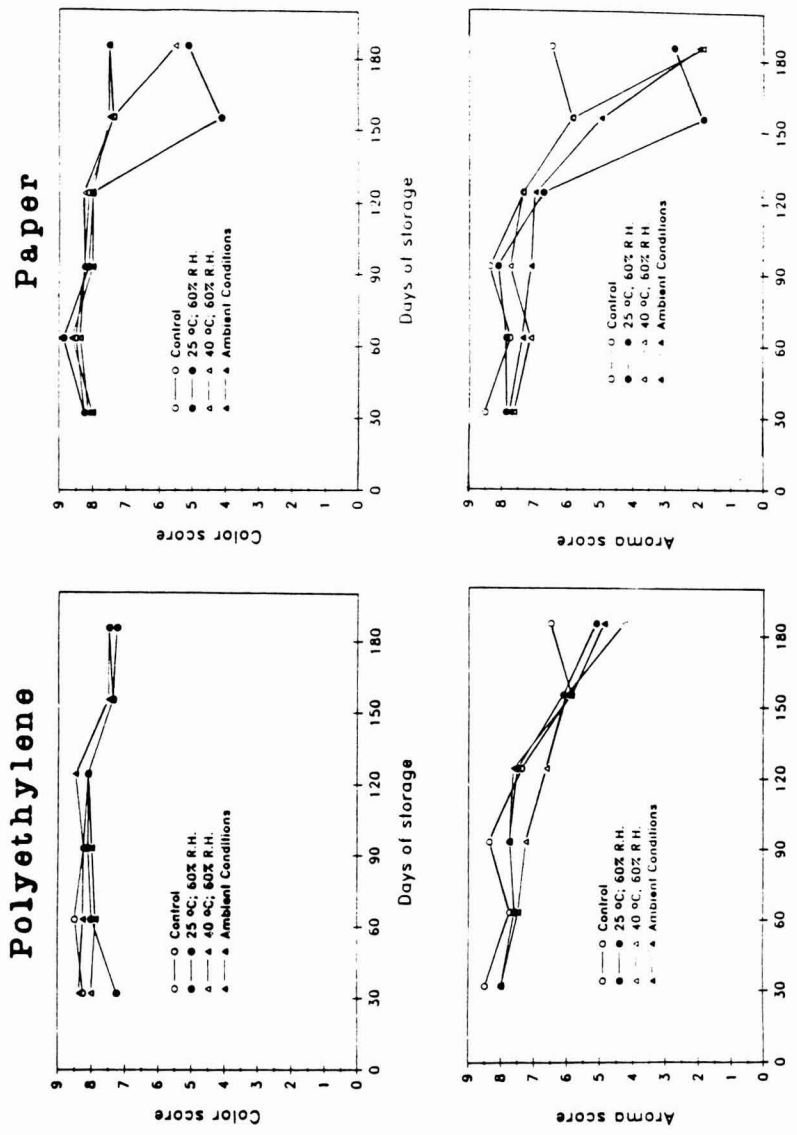

FIG. 6. EFFECT OF PACKAGING MATERIALS ON COLOR AND AROMA OF DRY MASA FLOUR STORED FOR 6 MONTHS AT DIFFERENT TEMPERATURES AND AT AMBIENT CONDITIONS

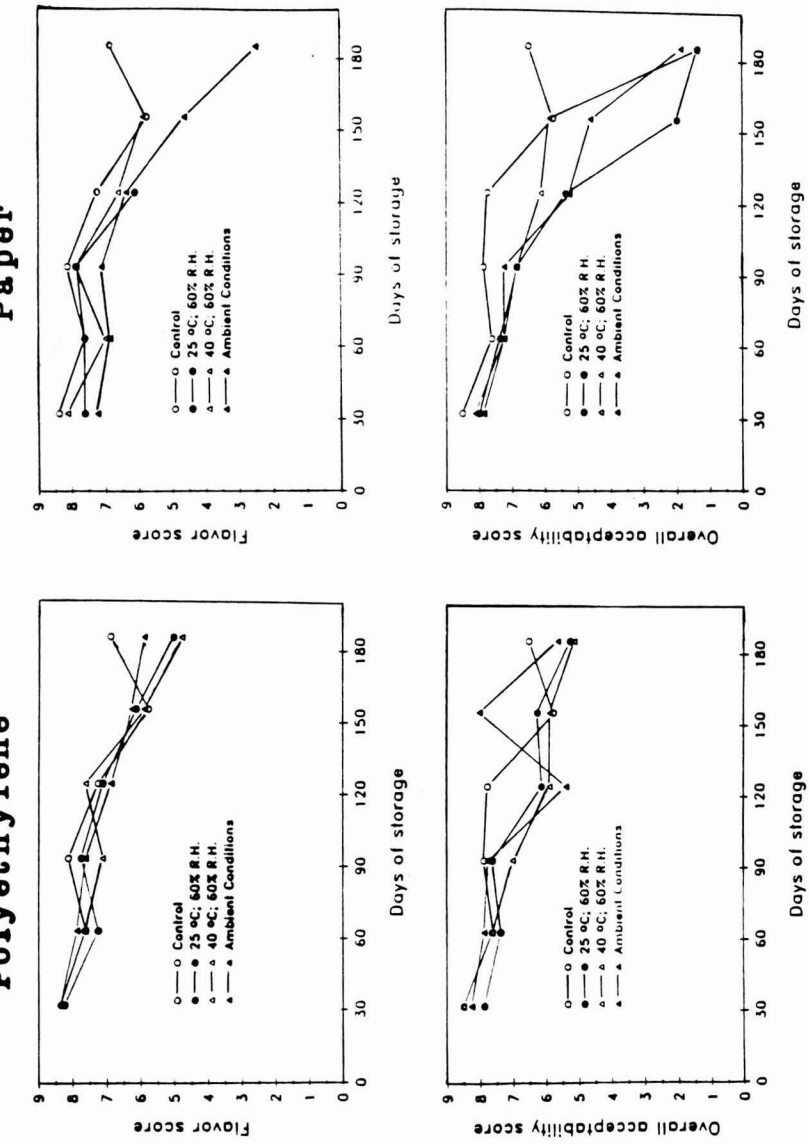

FIG. 7. EFFECT OF PACKAGING MATERIALS ON FLAVOR AND OVERALL ACCEPTABILITY OF DRY MASA FLOUR STORED FOR 6 MONTHS AT DIFFERENT TEMPERATURES AND AT AMBIENT CONDITIONS

For products packaged in polyethylene bags, a gradual reduction in flavor and overall acceptability was also observed. The scores were considerably reduced after 5 months storage when rancid-sour off-flavors were reported.

Tortillas. Sensory evaluation of tortillas manufactured from the dry masa flours packaged in paper bags was not performed due to the visible presence of molds. Results of sensory evaluations of the control tortilla and tortillas produced from masa flours packaged in polyethylene bags are presented in Table 4. Apparently, the most comparable tortillas to the control treatment were the ones produced out of dry masa flours stored at ambient conditions. Panelists reported that the treatment stored at 40°C had a rancid off-flavor and bad aroma resulting in a reduced general acceptability ($P < 0.05$) compared to the control treatment and counterparts stored at 25°C or under ambient conditions. Paredes and Mora (1983) also reported significant reductions in sensory scores in masa flour stored under accelerated conditions. Bothast *et al.* (1981) indicated that the sensory attributes (flavor and odor) of corn meal stored with the same moisture, deteriorated more rapidly at 34°C than at 25°C. Results of this study agree with their findings.

CONCLUSIONS

The shelf-life of dry masa flours mainly depended on the type of packaging system used and to a lesser extent on the environmental conditions. Dry masa flours can deteriorate faster in high relative humidity and temperature zones,

TABLE 4.
EFFECT OF STORAGE CONDITIONS ON THE SENSORY PROPERTIES OF TORTILLAS MANUFACTURED FROM DRY MASA FLOUR STORED FOR SIX MONTHS IN PLASTIC BAGS[a,b]

Sensory Attribute	Control[c]	Storage Conditions 25°C;60% R.H.	40°C;60% R.H.	Ambient[d]
Color	7.6a	7.9a	7.6a	7.4a
Odor	7.5a	7.4a	6.4b	7.0ab
Texture	7.3a	6.5ab	6.0b	6.0b
Overall Acceptability	7.4a	6.8ab	6.0b	6.6ab
Off-Flavors	---	---	Rancid	---

[a]Scores are averages of 30 observations. 1 = dislike extremely; 5 = neither like nor dislike and 9 = like extremely.
[b]Means within each row with different letters are statistically different at $P < 0.05$.
[c]Masa flour was stored in glass jars, flushed with nitrogen, covered with aluminum foil and refrigerated at 5°C.
[d]Average temperature of 36°C and 29% relative humidity.

such as tropical and subtropical areas of Mexico. A polyethylene package with a high resistance to tearing and low water vapor transmission rate is probably the most suitable to extend dry masa flour shelf-life. Its main disadvantage is that the package does not preserve its rectangular structure as the paper package does. Therefore, a good alternative is to design a package with an interior polyethylene film and exterior paper film. This material should be able to maintain both package structure and product shelf-life.

The main factor that affected product shelf-life was moisture content. The moisture content corresponding to an Aw of 0.8 was 15.2%. A high Aw was closely related with changes in fat acidity, pH, amylograph peak viscosity, mold counts, and sensory properties. At similar storage-packaging conditions, dry masa flour quality deteriorated more rapidly at 40°C than at 25°C. Therefore, a package which impedes the diffusion of environmental moisture can result in a substantial increase in shelf-life of dry masa flour.

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DEVELOPMENT OF KINETIC MODELS FOR METHIONINE DEGRADATION IN FORTIFIED SOYBEAN MODEL SYSTEMS

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ABSTRACT

Successful methionine fortification of soy protein may enhance its nutritional value. Methionine, however, is highly sensitive to processing conditions, hence the need for kinetic studies to optimize its retention. Kinetic models describing the effect of major compositional and processing parameters, such as moisture, protein and initial methionine content as well as temperature were developed in this investigation. Methionine degradation in these soy protein-containing systems appeared to follow a pseudo-first order reaction. Mathematical models clearly showed that all the parameters studied had a significant effect on the rate of methionine degradation. Statistical analysis of the kinetic data indicated that empirical equations can accurately describe methionine degradation as a function of the aforementioned parameters.

INTRODUCTION

A number of studies have evaluated the quality of soy protein and reported its amino acid composition (Campbell *et al.* 1985; Kolar *et al.* 1985; Harmon *et al.* 1969; Swaminathan 1967; Dies 1942; Horrath 1938). Soybeans have been found to have high protein content of good nutritional quality. Van Etten *et al.* (1959) compared the essential amino acid patterns of soy and whole egg protein and revealed a close similarity with the exception of the sulfur-containing amino acids, cysteine and methionine, both of which were lower in soy protein.

The nutritional quality of soy protein, although limited by its content of methionine, may be enhanced by addition of this amino acid (Hajós *et al.* 1988; Gaertner and Puigserver 1984; Chuah *et al.* 1983; Fomon *et al.* 1979; Graham

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et al. 1969; Panemangalore 1964). However, added free methionine may be inactivated or at least partially destroyed during processing or storage, resulting in less nutritionally available methionine. Oxidation of methionine model systems has been studied as a function of various agents such as hydrogen peroxide with or without the presence of iodide or selenite (Boonvisut *et al.* 1982) or ascorbic acid, dehydroascorbic acid, benzoic acid and ethanol (Aksnes and Njaa 1981). O'Keefe and Warthesen (1978) suggested Maillard browning as the main mechanism of free methionine destruction in glucose-containing model systems. Schleske and Warthesen (1982), however, found that methionine derivatives such as N-acetyl-methionine (NAM) may provide higher stability than methioinine with regard to Maillard browning, although decreased stability of NAM was observed in the presence of oxidized oil.

Strange *et al.* (1980) investigated losses of naturally occurring methionine in frankfurter emulsion model systems as a function of processing conditions and additives. Although processing conditions, including emulsification and smoking-cooking, had little influence on methionine retention, ingredient composition showed a significant effect on methionine content. In particular, the authors observed that the presence of high peroxide fats or sodium ascorbate contributed to the oxidation of methionine to methionine sulfoxide. On the other hand, addition of spices appeared to counteract the effect of the lipid peroxides, resulting in higher methionine retention. However, nitrite addition alone showed no effect but in combination with the spices, lowered methionine content.

Nielson *et al.* (1985) also showed a negative influence of oxidized lipids on stability of different amino acids, including methionine, lysine, cysteine and tryptophan, in a whey protein-methyl linolenate-water model system. Bioavailability studies of these systems, as measured by the rat assay, however, showed high bioavailability of the degradation product of oxidized methionine (methionine sulphoxide) as compared with those reaction products of lysine or tryptophan.

Wolf *et al.* (1981) studied the reaction order for free-lysine and methionine losses as a function of food composition. The authors found that protein, lipid, sugar and water activity had a significant effect on methionine degradation, described as following a first-order reaction. Strange (1984) found that oxidation of methionine in meat emulsion model systems as affected by hydrogen peroxide followed a pseudo-first-order reaction, and increasing pH from 5.8 to 7.2 increased the reaction rate. Other authors have studied supplemental free-methionine loss in food systems (Dunlap *et al.* 1974; Tufte and Warthesen 1979) and its bioavailability (Cabezas *et al.* 1982; Horn *et al.* 1968).

Fortification of foods with essential amino acids has been used to obtain balanced amino acid profiles and improved nutritional quality. While a balanced amino acid profile may be initially achieved by proper selection of proteins or

addition of free amino acids, processing and storage may destroy a substantial portion of essential amino acids such as methionine. For fortification to be effective, nutrient losses occurring during processing must be minimized and adjustments made in amino acid levels to account for their destruction. Loss of free methionine can be minimized by obtaining information on its stability in systems with varying ingredient composition and processing conditions. However, such an approach would require substantial experimental work, and the knowledge gained would be limited.

A predictive model can indicate how processes and compositions might be altered to maximize nutrient retention. In addition, kinetic models which describe destruction rates and their dependence on factors such as temperature could be useful in three categories: product improvement to minimize the loss of a quality factor (Lenz and Lund 1980 and Texeira *et al.* 1969), product development processes (Lund 1973) and shelf-life testing to predict stability during storage (Lee *et al.* 1977; Singh *et al.* 1975; Quast *et al.* 1972; Quast and Karel 1972).

A series of kinetic, heat and mass transfer studies are required to obtain a maximum of methionine retention during processing. Major parameters involved in methionine degradation should be examined and their effects isolated. Kinetic modeling and optimization, therefore, should be based on the effect of isolated parameters which are integrated to obtain an overall response for nutrient retention. The kinetic approach is based on establishing process rates of nutrient destruction, which can be generalized and correlated with environmental and compositional factors (Saguy and Karel 1979). Some general functional relationships have been found for major parameters as follows:

(1) Temperature. The most common approach to describe temperature-dependence of the deterioration rate of nutrients has been through the use of the Arrhenius equation. Since activation energy (E_a) is influenced by compositional factors, water activity, pH, etc., their effect must be considered (Saguy *et al.* 1978; Labuza 1973; Mizrahi *et al.* 1970; Tannenbaum *et al.* 1969).

Martens *et al.* (1981) calculated optimal sterilization conditions for minimum methionine degradation in a methionine model system (60–100°C) and in milk (115–128°C) and found activation energies of 29.9 and 30.8 kcal/mole, respectively.

Jokinen and Reineccius (1976) developed a mathematical model for the prediction of lysine losses during thermal processing of soy products. In this study, the average E_a was 28.5 kcal/mole. Kinetic information on methionine destruction as a function of temperature, however, is very limited.

(2) Moisture Content. Water in foods is important in controlling rates of degradation reactions. The means by which water would affect the rate of reaction is highly dependent on the mechanism involved, the composition of the product and the processing conditions. For instance, Mizrahi *et al.* (1970) established

correlations between moisture content and browning rates for dehydrated cabbage.

Tsao *et al.* (1978), in their studies at high temperature (115–185°C) indicated that the effect of moisture content (15–20%) on the rate of lysine degradation was much less significant than the effect of temperature. Wolf *et al.* (1981), on the other hand, found increased loss of either methionine or lysine with increasing water activity from 0.33–0.98 at 65 and 115°C.

Warmbier *et al.* (1976), in their studies in glycerol containing model systems, indicated an optimum rate of lysine degradation occurring at 0.4–0.5 water activity at 25–45°C, which is expected if lysine is destroyed via nonenzymatic browning.

(3) Initial Methionine Concentration. The initial content of methionine may have some effect on the degradation of methionine during processing. The initial molar ratio of free amino groups to reducing sugar compounds may affect the degree of reactivity in a given system (Lea and Hannan 1950; Warmbier *et al.* 1976). However, no significant work has been conducted to elucidate the effect of initial methionine content on its destruction.

(4) Protein Content. Protein has been reported as having a catalytic-like effect on the nonenzymatic browning reaction (Thompson *et al.* 1976). Furthermore, Wolf *et al.* (1981) showed that the presence of protein increased the rate of browning in methionine-fortified food systems containing varying quantities of protein between 0 and 20%, although Tufte and Warthesen (1979) found increased methionine protection against oxidation with increased protein concentration in oil-containing systems.

It is clear that degradation of nutrients may be reduced or increased, depending on the composition of the food systems and processing conditions. Therefore, results obtained for a product at a given set of processing conditions cannot be extrapolated to products with different compositions or to the same product subjected to different processing conditions. This problem may be circumvented, however, by using a mathematical approach based on kinetic studies, and separating and examining the effect of operating and compositional variables on nutrient retention.

MATERIALS AND METHODS

Preparation of Model Systems

A cellulose-based model system was selected to minimize the complexity encountered in real food systems and to isolate the effect of major parameters involved in methionine degradation. The model systems consisted of soy protein isolate (Ardex-F, ADM Co.), D-glucose (Mallinckrodt, Int.) DL-methionine

(Sigma Chemical Co.) and microcrystalline cellulose (Avicel PH-101, FMC Corp.)

The dry component composition of the model system was varied depending on the particular parameter under investigation or type of experiment being conducted. For the particular case of conditions typical of extrusion, compositions were selected to minimize interaction as well as to favor extrudability of the material. The following ranges of compositions were selected:

Ingredient	Low Temperature Studies	High Temperature Studies
microcrystalline cellulose	25–90%	35.5–55.5%
methionine	0.3–1%	0.3–0.7%
soy protein isolate	0–70%	40–60%
D-glucose	4–8%	4%

Unless otherwise specified, model systems were formulated containing 4% glucose.

Model systems were prepared by blending the different constituents for 15 min in a Sorvall® Omni Mixer Homogenizer (DuPont Co.) while immersed in an ice bath. An adequate amount of distilled deionized water was added to the dry ingredients to adjust the moisture content of the sample and mixed for 10 min. In systems looking at the effect of protein concentration, samples were prepared as mentioned using excess water, freeze-drying and humidifying the dried samples in a desiccator containing a saturated solution of cupric chloride (water activity of $\sim$ 0.65 at room temperature).

For the reaction kinetic studies, samples were placed in sealed screw cap vials (30 $\times$ 8 mm diameter) and heated either in a water bath (70–90°C) or an oil bath (110–150°C) and removed at predetermined intervals of time for analysis of methionine content.

Methionine Extraction Procedure

The sample extraction procedure used was a modification of the method developed by O'Keefe and Warthesen (1978). Exactly one gram of the sample was weighed and 10 mL of 10% methanol in water (v/v) were added and stirred for 20 min. Five milliliters of 40% trichloroacetic acid were then mixed with the sample solution, and the slurry filtered through a coarse fritted glass under vacuum. After the pH of the sample extract was adjusted to 9.0 with 40% NaOH, the extract was then quantitatively transferred to a 25 mL volumetric flask, and water was added to bring the extract to volume.

Methionine Determination

The free amino acid, methionine, extracted from the model systems was reacted with 5-dimethylamino-1-naphthalenesulfonyl chloride (dansyl chloride, Sigma Chemical Co.) according to the procedure of Bayer *et al.* (1976). Three milliliters of the sample extract, 0.5 mL of pH 9.0 borate buffer (0.1 M) and 1.5 mL of 10 mM dansyl chloride in acetone were reacted for one hour at 40°C to form the dansyl derivative of methionine. Samples were then filtered through a 0.4 μm Millipore membrane filter, followed by separation and quantification of methionine, accomplished by high pressure liquid chromatography (HPLC).

A Beckman Model 324 HPLC system including a Model 421 microprocessor system controller, two solvent metering pumps (Models 100A and 110A), a Model 210 20μL injection valve and a Model 155 variable wavelength detector set at 254 nm were used to quantitate methionine concentration. Dansyl derivatives of methionine were analyzed by reverse phase chromatography using a μ-Bondapak C_{18} as the stationary phase and a mixture of 24.5% acetonitrile and 75.5% 0.01M phosphate buffer (pH 7.0) as the mobile phase.

Experimental Design

The initial part of this investigation involved conducting a series of isothermal kinetic studies in order to obtain a mathematical model describing methionine degradation and in an effort to elucidate mechanisms of interaction. Major parameters involved in methionine degradation were examined and their effects isolated and determined with the aid of model systems. Rate constants (k) were established as a function of only one independent variable at a time:

k vs. mc (moisture content)
k vs. T (temperature)
k vs. pc (protein content)
k vs. imc (initial methionine concentration)

After models for each independent variable were defined, an overall mathematical model for methionine degradation was developed as a function of all major parameters. Functional relationships were generated by fitting appropriate functions to the partial response and then multiplying them together to obtain an overall response.

Isothermal methods were also employed to obtain kinetic models for methionine degradation at conditions characteristic of extrusion which is a high-temperature short-time process performed at moderate moisture content. Experiments considering the effect of each parameter on methionine degradation were conducted at various levels of its concentration with other independent variables remaining constant. Models for methionine degradation as a function of each parameter were developed based on the experimental data.

Five-dimensional analysis was required to obtain an overall response, since the degradation of methionine in this investigation is a function of four independent variables (mc, T, pc, imc). Although, in general, a larger number of observations would result in a higher degree of accuracy in mathematical models describing the kinetics of methionine degradation, the amount of experimental data required may be reduced by choosing an adequate experimental design.

The dependent variable (k) was studied as a function of two independent variables by multiplying two isolated functions (k vs. mc and k vs. pc):

$$k = f(mc, pc) \qquad \text{(Eq. 1)}$$

To find this combined effect on the degradation of methionine, different moisture levels in the model system were investigated at different protein concentrations. A mathematical model for three independent parameters was then established by multiplying one more response to yield a function:

$$k = f(mc, pc, imc) \qquad \text{(Eq. 2)}$$

Finally a functional relationship to yield an approximate description of the overall response was generated by fitting appropriate functions to the partial responses:

$$k = f(mc, T, imc, pc) \qquad \text{(Eq. 3)}$$

Statistical Analysis

Kinetic models were established by using theoretical, empirical and statistical considerations. Starting with approximate values of the constants, least square fitting was used to estimate the best values of these constants with the aid of several programs on an IBM 4341 computer. Two types of least squares fitting were used. Stepwise linear regression programs were employed for linear models, while for nonlinear models, nonlinear least squares fitting was used in the Statistical Analysis System (SAS). Although the nonlinear models in general require more computation time, such models often give significantly better fitting.

RESULTS AND DISCUSSION

Effects of Major Parameters on Methionine Degradation

The effects of major parameters involved in methionine degradation were isolated and examined with the aid of model systems.

Moisture Content. The retention of methionine was measured as a function of moisture content at constant temperature keeping the other independent variables constant. The reaction constant was determined from the equation:

$$-\frac{dc}{dt} = kc^n \qquad \text{(Eq. 4)}$$

Where c = concentration of methionine,
n = order of the reaction (found to be 1 for our systems at 84°C) and
t = reaction time

It was observed that at an initial methionine concentration of 1.0%, a maximum of methionine degradation occurred at a moisture content of approximately 8% (0.6–0.65 water activity at room temperature). The rate of methionine degradation increased with an increase in water content from 6 to 8%, while lower rates of degradation were observed as water content was increased from 8 to 25% (Fig. 1). Similar trends were observed when the initial level of methionine concentration was 0.5%.

These effects may result from water's dual role as a solvent and as a product of the reaction. At low water activity, the limiting factor is inadequate mobility of the reactants and, therefore, an increase in water content of the system accelerates the reaction. Above a certain level of moisture content, however, a dilution effect becomes the predominant factor. In addition, since water is a product of condensation in nonenzymatic browning reactions, it may contribute to hinder the reaction by "product inhibition."

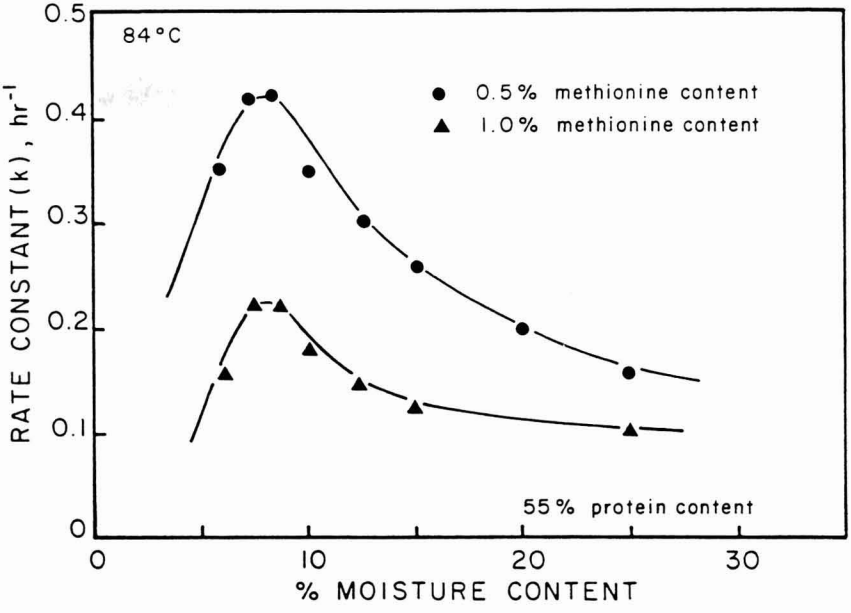

FIG. 1. DEPENDENCY OF METHIONINE DEGRADATION ON MOISTURE CONTENT IN MODEL SYSTEMS (55% PROTEIN, 4% GLUCOSE) AT 84°C FOR TWO DIFFERENT INITIAL METHIONINE CONCENTRATIONS

Protein Content. Protein showed a catalytic-like effect on the degradation of methionine for concentrations up to 25%; however, any increase in protein above 25% reduced the reaction rate (Fig. 2). Overall, the effect of protein was found to be complex and dependent on reducing sugar concentration. It should be kept in mind that native 7S and 11S polypeptides of soy protein are compactly folded so that their internal hydrophobic amino acids are not accessible. However, heat treatment causes dissociation of glycinin and subsequent unfolding as a result of disulfide bond cleavage. Most of the basic and hydrophobic amino acids in the 11S fraction become accessible following unfolding and denaturation.

In general, the specific role of protein in methionine degradation is rather difficult to elucidate. Based on our experimental results, it seems that at low protein concentration, below 25%, protein incorporation accelerates the destruction of methionine. It is considered that the hydrophilic regions of soy protein may bind to the hydroxyl groups of sugar compounds through hydrogen bonding. Furthermore, denatured protein will expose a number of hydrophobic groups, which make it possible to form hydrophobic bonds among hydrophobic amino acids. Therefore, protein interaction both with hydroxyl groups of reducing sugar compounds (due to hydrogen bonding) and with the hydrophobic groups of

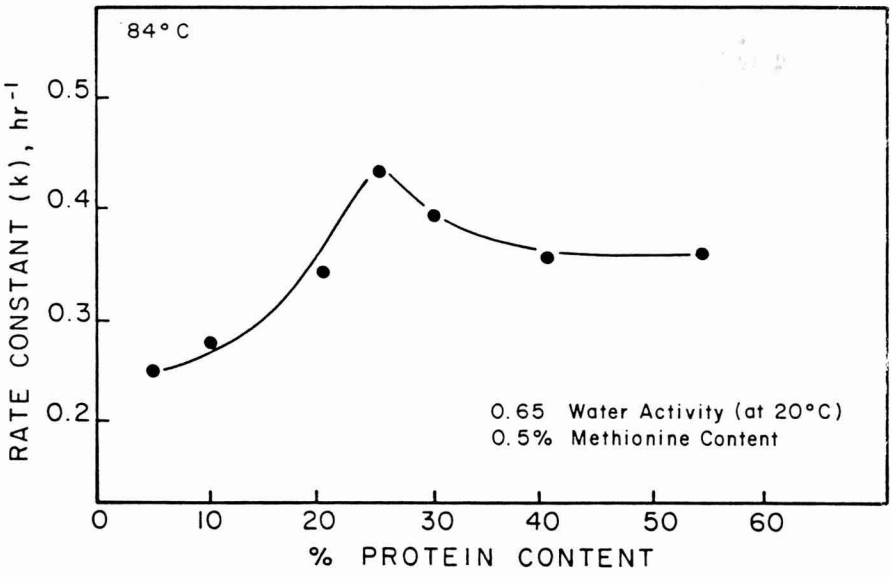

FIG. 2. DEPENDENCY OF METHIONINE DEGRADATION ON PROTEIN CONTENT IN MODEL SYSTEMS CONTAINING 4% GLUCOSE AT 84°C
(initial methionine concentration = 0.5%)

methionine (due to hydrophobic bonding) is feasible. This reaction may contribute to sterically push methionine and the reducing sugar closer together, favoring their interaction.

In addition, protein may bind with water due to hydrogen bonding. The water binding capacity of systems may increase as the protein content and the degree of denaturation increase. Heat treatment of the protein will expose additional sites for water binding by dissociation and unfolding. Therefore, the addition of protein will result in a higher degree of water binding controlling water activity in systems undergoing nonenzymatic browning where water is a product of the condensation reaction.

However, since heat treatment of proteins will expose their internal amino acids such that they may be attacked by reducing sugar molecules, protein and methionine will compete for the sugar molecules, and the rate of methionine degradation may decrease, depending upon protein concentration. It was observed that the rate of methionine degradation was greatly decreased as protein content increased in systems containing 4% glucose (Fig. 3). In order to inves-

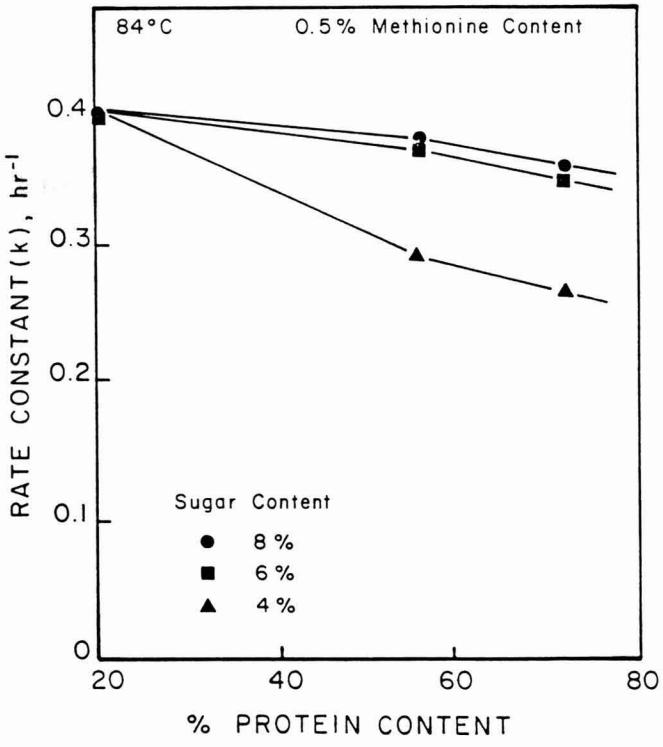

FIG. 3. COMBINED EFFECT OF PROTEIN AND GLUCOSE CONTENT ON METHIONINE DEGRADATION IN MODEL SYSTEMS AT 84°C (imc = 0.5%)

tigate the influence of protein-sugar interaction on the browning rate, the glucose concentration was increased from 4% to 8% in the system. Although there was no significant difference between the 6 and 8% glucose systems, the rate of methionine degradation greatly increased as sugar concentration increased from 4 to 6% at high protein levels. It is considered that at low concentrations of glucose (4%), the protein may have a protective mechanism on methionine degradation by preventing its participation in the browning reaction and by favoring protein-sugar interaction. The competition between protein and methionine for sugar molecules seems to reduce the rate constant. In fact, the effect of protein decreased as the glucose concentration was increased to levels 6% and higher, since sufficient sugar may be available for both methionine and protein interactions.

The influence of protein on methionine degradation may also be affected by the presence of lipids due to possible lipid-protein interaction. Since the isolated soy protein utilized in these experiments contained 3.4% oil of which 0.5% was free fatty acids, the oil content in the system increased with the level of protein incorporation. On the other hand, the presence of oil in the system may increase the methionine degradation rate, since carbonyl compounds and peroxides formed during lipid oxidation are able to react with amino acids via the Strecker degradation. The extent of this interaction may or may not be significant since protein may protect methionine from lipid oxidation (Tufte and Warthesen 1979). Lipid radicals may attack the protein and produce protein radicals which would cause the protein to polymerize. Protein polymerization would eliminate free radicals and reduce the extent of lipid oxidation. Our systems formulated containing added oil concentrations up to 8%, indicated that the effect of lipids on methionine degradation was not significant, possibly due to lipid-protein interaction or relatively low rates of lipid oxidation, which would minimize the presence of free radicals in the system.

The pH levels of the systems containing different amounts of protein and methionine were measured to investigate the effect of protein concentration on pH. Our systems showed relatively constant pH for levels of protein incorporation ranging between 20 and 70%, most likely due to the buffering action of proteins. Therefore, this would imply that the influence of protein on methionine degradation is not related to the effect of pH on the browning reaction, since the pH of the systems remained relatively constant around pH 6.8 for the entire protein concentration range studied.

In conclusion, the addition of protein increases methionine degradation at low protein levels (0–25%), possibly due to closer proximity of methionine to glucose caused by interactions of methionine and reducing sugar with protein. However, the inhibitive effect of protein at high protein levels (25–70%) may be explained mostly by protein-sugar interaction which results in less available reducing sugar for methionine.

Initial Methionine Concentration. The rate of degradation was greatly influenced by the initial concentration of methionine (Fig. 4). The rate constant increased as the concentration of methionine decreased from 1% down to 0.5% and unexpectedly decreased below this concentration level. Previous studies have indicated that the browning rate is significantly influenced by the initial molar ratio of reducing sugars to amino acids, since these components are the primary reactants (Warmbier *et al.* 1976). The authors observed that the rate of degradation increased linearly as the molar ratio of glucose to available lysine increased from one half to five due to the higher relative concentration of reducing sugars available for interaction with lysine. Above this ratio of concentrations, the rate constant did not change possibly due to a saturation effect of glucose in the system. In our investigation, although the trends observed at high concentrations of methionine seemed to follow those reported for lysine by Warmbier and his coworkers, at low methionine concentrations a totally different pattern was obtained. In this system containing an excess of glucose, the rate of methionine degradation should have remained unchanged in the range from 6:1 to 11:1 glucose to methionine ratios. This overall phenomenon may be explained by considering the effect of protein. Added protein may bind to both methionine and glucose, therefore, promoting methionine degradation on the basis of closer proximity of the reactants. At concentrations of methionine below 0.5%, it seems that a decrease in rate of methionine degradation may result from diminishing

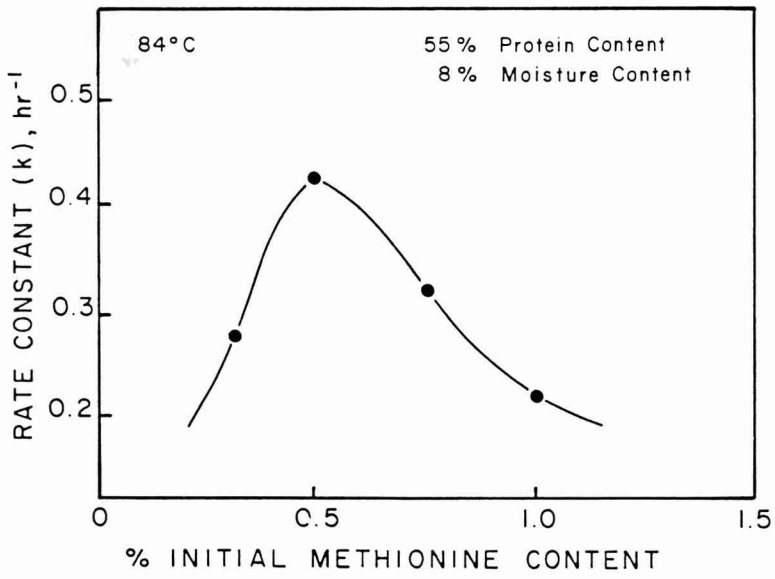

FIG. 4. EFFECT OF INITIAL METHIONINE CONCENTRATION ON METHIONINE DEGRADATION IN MODEL SYSTEMS (55% protein/8% moisture content/4% glucose) at 84°C

the relative concentration of methionine available for reaction with glucose. Based on our previous results, protein exerts an overall protective mechanism on methionine degradation. Since simultaneous protein-glucose and methionine-glucose interactions occur in this food system, their relative concentrations are important to determine whether free or protein-amino acids will undergo preferential attack by reducing sugar molecules.

Since the rate of methionine degradation was greatly affected by initial concentration of methionine, the degradation of methionine was found to follow a pseudo first-order reaction.

Temperature. Model systems containing 8% moisture content were heated in water baths at three different temperatures: 70°, 80° and 90°C. A common kinetic assumption is that temperature dependence of deterioration rates can be expressed by the Arrhenius equation. In this investigation, it was found that the data followed an Arrhenius correlation; the activation energy was determined to be 18.3 kcal/mole (Fig. 5). It is clear that the activation energy may vary substantially if the reaction mechanism changes with temperature; therefore, the range of validity and influence of other factors on the activation energy must be considered.

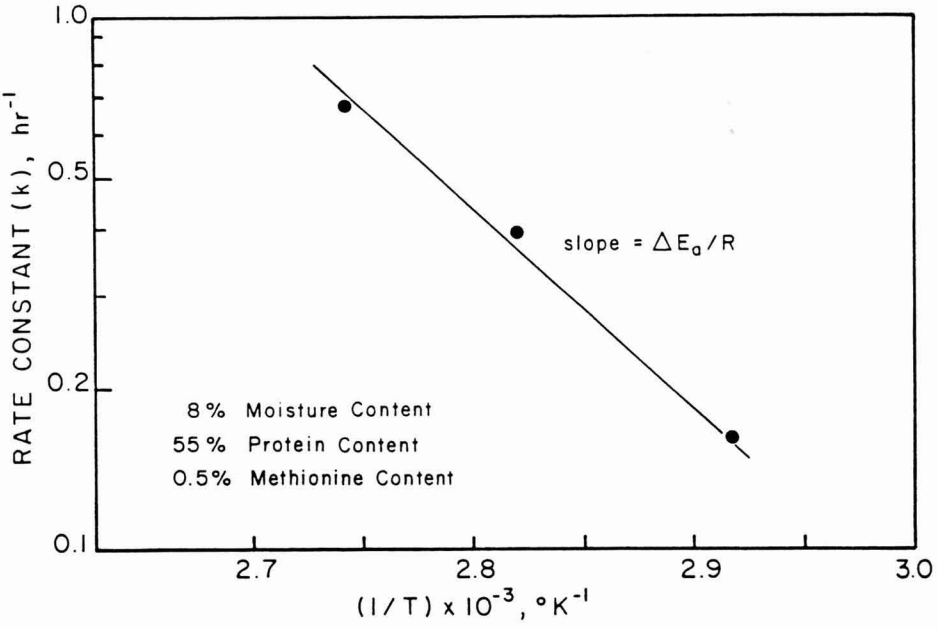

FIG. 5. DEPENDENCY OF METHIONINE DEGRADATION ON TEMPERATURE IN MODEL SYSTEMS (55% PROTEIN/8% MOISTURE/4% GLUCOSE) WITH INITIAL METHIONINE CONCENTRATION = 0.5%

Mathematical Modeling

Models of One Independent Variable. Models for methionine degradation were developed as a function of only one parameter at a time such as moisture content, protein content and initial methionine concentration (Table 1). Conditions selected for this study were within the range of those in an extrusion process for protein texturization.

Moisture Content. Rate constants at 140°C were determined at various moisture contents in systems with 0.5% initial methionine concentration and 40% protein content. The rate of methionine degradation decreased with an increase in water content from 10 to 30% (Fig. 6). Five linear regression programs were employed to correlate this experimental data and determine their accuracy in describing the effect of moisture (Table 1).

Analysis of our computed values showed excellent agreement between predicted and experimental results as described by Eq. (5) with a r^2 of 0.99 and a low s^2. In Table 1, the estimates of the constants for the best equation (Model 1) are shown with their standard deviations.

Protein Content. Experiments were conducted at four levels of protein content at 140°C keeping other variables constant in order to investigate the influence of protein on the nonenzymatic browning reaction (Fig. 7). Several models were tested with their corresponding r^2 being summarized in Table 1. Inspection of plots describing the experimental results suggested that a good linear relationship as described by Eq. (6) can be obtained between pc/k and pc. Model (2) satisfactorily represents the experimental results, with low s^2 and high r^2; its estimated constants are represented in Table 1.

Initial Methionine Concentration. Experiments were conducted at 140°C in model systems with 40% protein, 15% moisture content and four levels of methionine to isolate the effect of initial methionine concentration on its rate of degradation. In a plot of rate constant versus imc (Fig. 8), the rate constant increased as the concentration of methionine increased from 0.3 to 0.5%.

However, the rate of degradation after passing through a maximum at 0.5% decreased as the concentration of methionine increased. This trend suggested that high degree polynomials may be required to predict this behavior. As may be seen in Table 1, the value of s^2 was calculated to be 0.17 for a second degree polynomial (Eq. 12) and 0.00 for a third degree polynomial (Eq. 13). Addition of the term imc^3 resulted in improved accuracy if a more complicated model could be tolerated with the presence of four constants (Model 3).

Temperature. In general, extrusion processing may be conducted at high temperatures ranging approximately from 110° to 170°C. To describe the mechanism of the methionine degradation reaction at high temperatures, rates of methionine degradation were examined at 110°, 130°, 140° and 150°C. Figure 9 shows the dependency of methionine degradation on temperature. This tendency followed

TABLE 1.
MODELS FOR ONE INDEPENDENT VARIABLE AND ITS EFFECT ON METHIONINE DEGRADATION.

Trial Model		r^2	s^2	Equation No.	Constant	Value	Standard Deviation
1. Effect of Moisture Content (mc):					[a]Values of Constants for Selected Model (1): mc/k = P1 + P2(mc)		
linear:	k = P1 + P2(mc)	0.96	0.045	(1)			
	$k = P1 + P2(mc) + P3(mc)^2$	0.97	0.066	(2)	P1	-0.0109	0.00316
	log k = P1 + P2 log(mc)	0.97	0.0002	(3)	P2	0.2403	0.0156
	lnk = P1 + P2(mc)	0.97	0.0017	(4)			
	mc/k = P1 + P2(mc)	0.99	0.00005	(5)[a]			
2. Effect of Protein Content (pc):					[b]Values of Constants for Selected Model (2): pc/k = P1 + P2(pc)		
	pc/k = P1 + P2(pc)	0.99	0.00005	(6)[b]			
linear:	Lnk = P1 + P2(pc)	0.85	0.09	(7)	P1	-0.0125	0.0064
	$k = P1 + P2(pc) + P3(pc)^2 + P4(pc)^3$	1.0	0.0	(8)	P2	0.2121	0.016
non-linear:	k = P1·Exp(P2·pc)		0.064	(9)			
3. Effect of Initial Methionine Concentration (imc):					[c]Values of Constants for Selected Model (3): $k = P1 + P2(imc) + P3(imc)^2 + P4(imc)^3$		
$Lnk = P1 + P2(imc) + P3(imc)^2$		0.95	0.0043	(10)	P1	1.325×10	0.0
$k = P1 + P2(imc)^2 + P3(imc)$		0.88	0.25	(11)	P2	-8.220×10^2	0.0
$k = P1 + P2(imc) + P3(imc)^2$		0.92	0.17	(12)	P3	2.250×10^4	0.0
$k = P1 + P2(imc) + P3(imc)^2 + P4(imc)^3$		1.0	0.0	(13)[c]	P4	-1.792×10^5	0.0

k = rate constant of non-enzymatic browning reaction

r^2 = measurement of variation in the dependent variable as accounted by the model. The larger the value of r^2, the better the model's fit.

s^2 = the mean square for error, estimate of the variance of the true residuals.

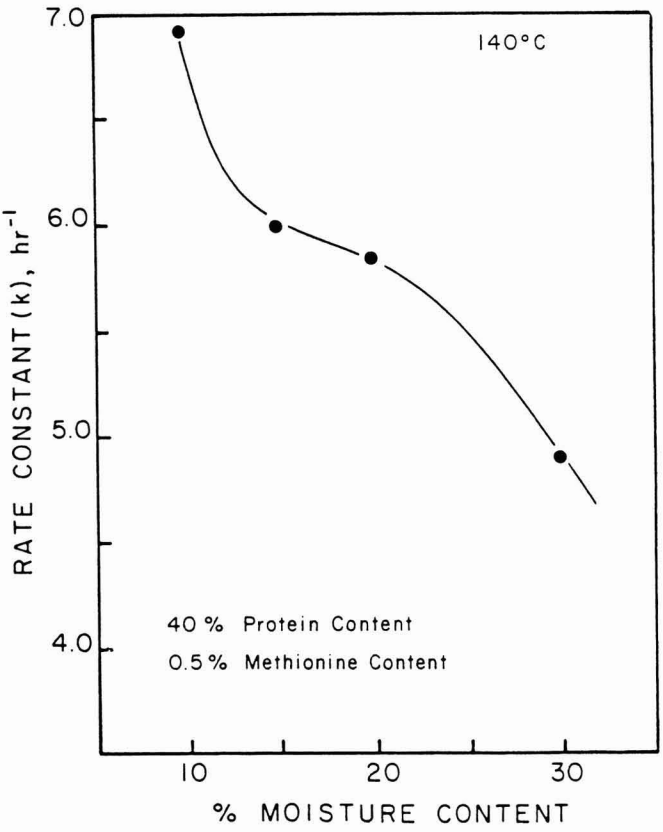

FIG. 6. DEPENDENCY OF METHIONINE DEGRADATION ON MOISTURE CONTENT IN MODEL SYSTEMS (40% PROTEIN/4% GLUCOSE/0.5% INITIAL METHIONINE CONCENTRATION) AT 140°C

an Arrhenius type of correlation with an activation energy calculated to be 17.5 kcal/mole. Thus, a model for temperature effect would be as follows (Model 4):

$$\text{Lnk} = \text{Lnk}_o - \frac{E_a}{R} \cdot \frac{1}{T} \qquad \text{(Eq. 14)}$$

Where: k = reaction rate constant
k_o = collision factor
R = gas constant
T = absolute temperature
E_a = activation energy

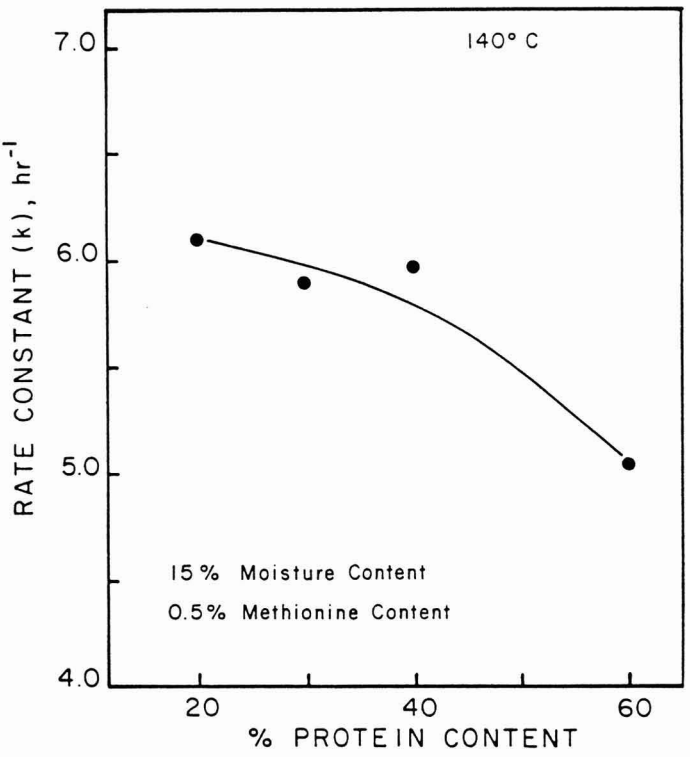

FIG. 7. DEPENDENCY OF METHIONINE DEGRADATION ON PROTEIN CONTENT IN MODEL SYSTEMS (15% MOISTURE/4% GLUCOSE/0.5% INITIAL METHIONINE CONCENTRATION) AT 140°C

Models of Two Independent Variables. A statistically designed factorial experiment was chosen to examine the combined effects of three independent variables in model systems: water content, protein content and initial methionine concentration.

Rates of methionine degradation at 140°C were determined at the following values of the independent variables to investigate the combined effects of two parameters at a time.

Moisture content: 10, 15, 20, 30 (%)

Protein content: 20, 40, 60 (%)

Initial methionine concentration: 0.3, 0.5, 0.7 (%)

Effects of Protein and Moisture Content. In order to examine the interactions between moisture and protein in the methionine degradation reaction, a 4 × 3 factorial experiment was conducted at 140°C. The reaction rate for the destruction of methionine was found to decrease as protein content increased from 20 to

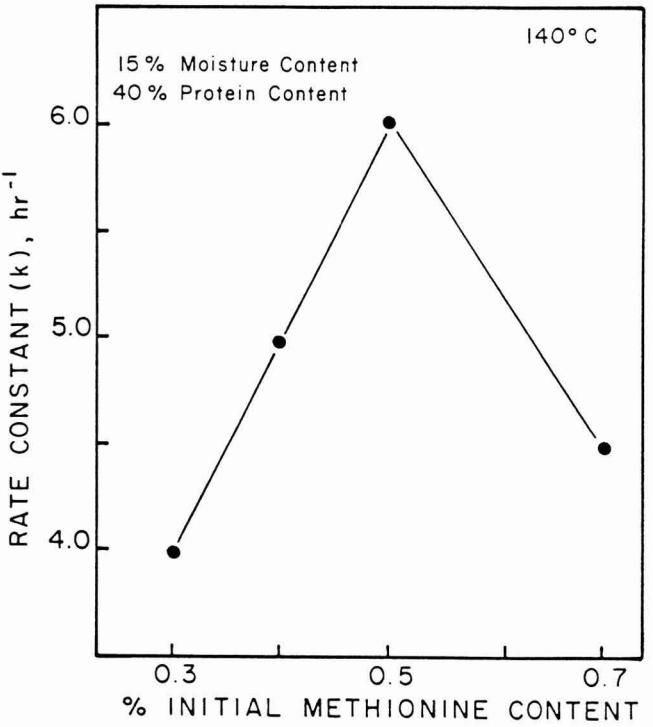

FIG. 8. DEPENDENCY OF METHIONINE DEGRADATION ON INITIAL METHIONINE CONCENTRATION IN MODEL SYSTEMS (40% PROTEIN/15% MOISTURE/ 4% GLUCOSE) AT 140°C

60% and also to decrease as moisture content increased from 10 to 30% (Fig. 10). When the main effects and interactions were determined by analysis of variance of the results, the linear effects of both parameters were found to be significant with no indication of curvature. The interaction between protein and water content in this experiment was not significant either in the linear or in the quadratic components. Based partially on knowledge gained from the statistical analysis and the development of models with one independent variable, models with two independent variables were generated. Two basic types of least square fitting were used: linear and nonlinear models. Results for different models were compared and the most suitable were chosen (Table 2). Model (5) was found to be useful for further application in models of three independent variables from the point of view of less number of constants (Table 2). Even though Eq. (17) is complicated by the large number of constants, it was felt that this model was also useful due to a significant reduction in s^2.

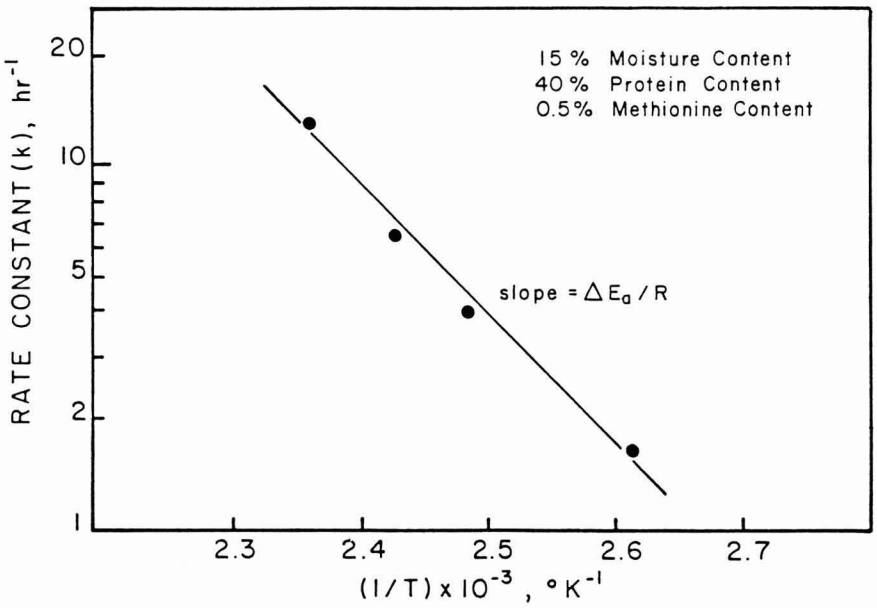

FIG. 9. DEPENDENCY OF METHIONINE DEGRADATION ON TEMPERATURE IN MODEL SYSTEMS WITH 40% PROTEIN/15% MOISTURE/4% GLUCOSE/ 0.5% INITIAL METHIONINE CONTENT

Effects of Moisture Content and Initial Methionine Concentration. A 4 × 3 factorial experiment was conducted at 140°C to investigate the interactions between moisture and methionine in the nonenzymatic browning reaction. The reaction rate decreased as moisture content increased from 10 to 30%, showing a maximum at 0.5% initial methionine concentration (Fig. 11). The reaction rate decreased both above and below the 0.5% initial methionine concentration independent of moisture content. The significance of new effects and interactions was determined by the analysis of variance. Only the linear component of the moisture content effect and the quadratic effect of the initial methionine concentration were significant.

Mathematical models of two independent variables may be developed by integrating two individual models of one independent variable. However, the correlation of individual models should be determined to establish an appropriate model as a function of the two parameters by correlating the data with the aid of a computer.

Both linear and nonlinear models were established based on the isolated effect of moisture and methionine content on methionine degradation. Possible models are summarized and compared in Table 2. A large reduction in s^2 was obtained

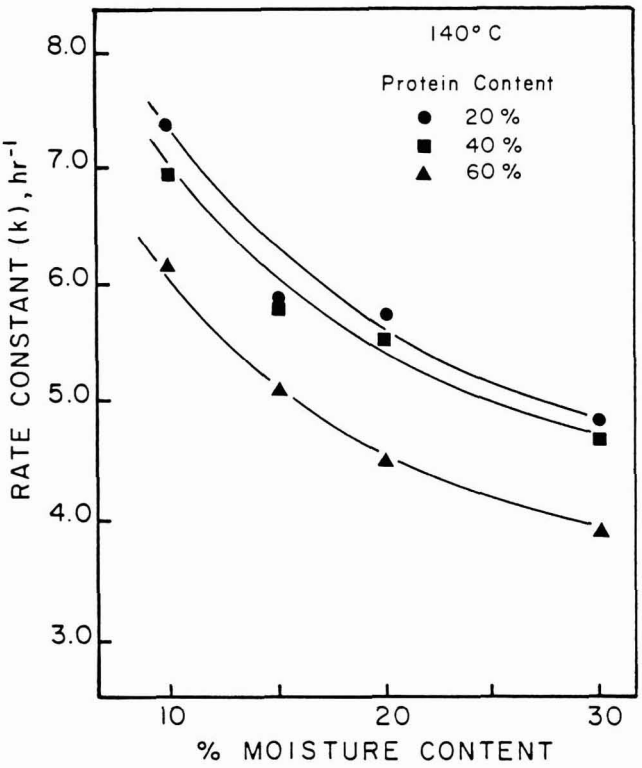

FIG. 10. EFFECT OF MOISTURE CONTENT ON METHIONINE DEGRADATION AT 140°C IN MODEL SYSTEMS WITH 4% GLUCOSE, VARYING PROTEIN CONTENT AND AN INITIAL METHIONINE CONCENTRATION = 0.5%

with the application of a nonlinear model (Eq. 23). Values of the constants for this equation (Model 6) were estimated and are summarized in Table 2 with their corresponding standard deviations.

Effects of Protein and Initial Methionine Concentration. Although the addition of protein to systems may result in some cases in higher rate constants of methionine degradation due to the ability of protein to facilitate sugar-amino acid interaction, beyond 20% protein, the rate constant decreased as protein content increased possibly due to protein-sugar interactions (Fig. 12). Initial methionine concentration is another major parameter that should be considered as affecting the kinetics of amino acid degradation.

In preliminary experiments carried out in systems containing 40% protein, a maximum rate constant was obtained at 0.5% methionine content. For the conditions investigated, degradation rates decreased above and below 0.5% initial methionine concentration. To establish whether or not this same trend would

TABLE 2.
MODELS FOR TWO INDEPENDENT VARIABLES AND THEIR EFFECTS ON METHIONINE DEGRADATION.

Trial Model	r^2	s^2	Equation No.	Constant	Value	Standard Deviation
1. Combined Effect of Protein Content (pc) and Moisture Content (mc):				[a]Values of Constants for Selected Model (5): $Lnk = mc \cdot pc/[P1 + P2(pc)]$		
Linear:						
$k = P1 + P2(mc)^{\frac{1}{2}} + P3(mc) + P4(pc)^{\frac{1}{2}} + P5(pc)$	0.96	0.050	(15)			
$k = P1 + P2(mc) + P3(mc) + P4(pc)^2 + P5(pc)^2$	0.96	0.060	(16)	P1	-0.0036	0.014
$Lnk = P1 + P2(mc) + P3(mc)^2 + P4(pc) + P5(mc)(pc) + P6(mc)^2(pc)$	0.97	0.020	(17)	P2	0.1427	0.050
Non-linear:						
$K = P1 \cdot Exp(P2 \cdot mc) + pc/[P3 + P4(pc)]$	-	0.070	(18)			
$Lnk = mc \cdot pc/[P1 + P2(pc)]$	-	0.030	(19)[a]			
2. Combined Effect of Initial Methionine Concentration (imc) and Moisture Content (mc):				[b]Values of Constants for Selected Model (6): $k = P1 \cdot Exp[P2(mc)] + imc/[P3 + P4 \cdot imc]$		
Linear:						
$k = P1 + P2(mc) + P3(imc) + P4(mc)^2 + P5(imc)^2$	0.87	0.140	(20)	P1	6.650	0.243
$k = P1 + P2(mc) + P3(imc) + P4(mc)(imc) + P5(mc)^2 + P6(imc)^2$	0.87	0.156	(21)	P2	-1.830	0.202
$k = P1 + P2(mc) + P3(imc) + P4(mc)(imc) + P5(mc)^2 + P6(imc)^2 + P7(imc)^2(mc)^2$	0.87	0.180	(22)	P3	-1.051	0.591
				P4	21.909	3.830
Non-linear:						
$k = P1 \cdot Exp[P2(mc)] + imc/(P3 + P4(imc)]$	-	0.052	(23)[b]			
$Lnk = (mc)(imc)/[P1 + P2(imc)]$	-	0.580	(24)			
3. Combined Effect of Initial Methionine Concentration (imc) and Protein Content (pc):				[c]Values of Constants for Selected Model (7): $k = P1 + P2(pc) + P3(imc) + P4(pc)^{\frac{1}{2}} + P5(imc)^{\frac{1}{2}} + P6(pc)(imc) + P7(pc)^{\frac{1}{2}}(imc)^{\frac{1}{2}}$		
Linear:						
$k = P1 + P2(pc) + P3(pc)^2 + P4(imc) + P5(imc)^2$	0.96	0.031	(25)			
$k = P1 + P2(pc) + P3(imc) + P4(pc)(imc) + P5(pc)^2 + P6(imc)^2 + P7(pc)^2(imc)^2$	0.98	0.033	(26)	P1	-2.305	1.21
				P2	1.505	0.11
				P3	-346.897	9.84
				P4	-23.999	2.46
				P5	96.465	7.16
$k = P1 + P2(pc) + P3(imc) + P4(pc)^{\frac{1}{2}} + P5(imc)^{\frac{1}{2}} + P6(pc)(imc) + P7(pc)^{\frac{1}{2}}(imc)^{\frac{1}{2}}$	0.99	0.025	(27)[c]	P6	-315.628	20.53
				P7	175.511	10.93
Non-linear:						
$k = P1 \cdot Exp(P2 \cdot pc) + imc/(P3 + P4 \cdot imc)$	-	0.180	(28)			
$Lnk = pc \cdot imc/(P1 + P2 \cdot imc)$	-	0.250	(29)			

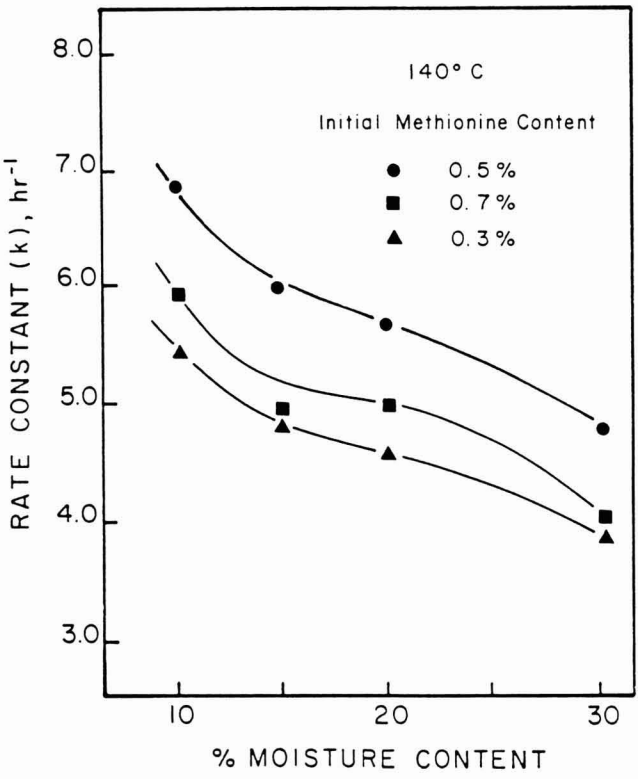

FIG. 11. EFFECT OF MOISTURE CONTENT ON METHIONINE DEGRADATION AT 140°C IN MODEL SYSTEMS WITH 40% PROTEIN, 4% GLUCOSE AND VARYING INITIAL LEVELS OF METHIONINE

follow at extrusion conditions, a 3 × 3 factorial experiment was conducted to characterize the interaction between methionine and protein.

The main effects of methionine and protein were found significant by analysis of variance. The quadratic components of initial methionine content were significant, indicating a falling off in the response in systems containing methionine above and below 0.5% concentration. However, the main effect of the protein can be defined as a linear relationship, showing an insignificant quadratic effect.

Two types of least square fitting were used to establish the combined model as a function of protein and initial methionine concentration based on information obtained in the statistical analysis and the development of models of one independent variable. For linear models, polynomial expressions were established (Table 2). The large increase in r^2 when the interaction effects were introduced into Eq. (25) produced a better fitting equation (Eq. 27). However, a more complicated model with seven constants would have to be tolerated. A second

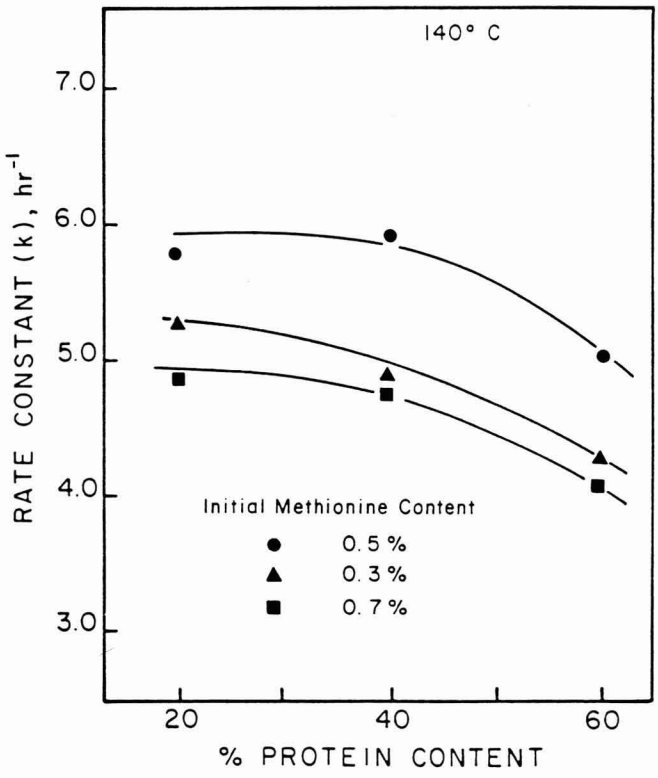

FIG. 12. EFFECT OF PROTEIN CONTENT ON METHIONINE DEGRADATION AT 140°C IN MODEL SYSTEMS WITH 15% MOISTURE, 4% GLUCOSE AND VARIOUS INITIAL LEVELS OF METHIONINE

degree polynomial with interactions (Eq. 27) is a possible model with a mean square error of 0.025, even if this model generates seven constants (Model 7, Table 2).

Models of Three Independent Parameters. A 4 × 3 × 3 factorial experiment was conducted in model systems in order to examine the combined effect of moisture content, initial methionine concentration and protein content on methionine degradation. The rate of methionine degradation was determined as a function of these variables, based on the models of one and two independent variables.

The simplest empirical models were polynomials and, for several independent variables, products of polynomials. As a term of comparison, a linear model containing the terms pc, imc, mc, their squares and all products of these were investigated (Table 3). Nonlinear models were established based on the knowledge gained from the development of models of two independent parameters.

TABLE 3.
MODELS FOR THREE INDEPENDENT VARIABLES AND THEIR EFFECTS ON METHIONINE DEGRADATION.

	Trial Models	r^2	s^2	Equation No.
Linear:	$k = P1 + P2(pc) + P3(mc) + P4(imc) + P5(pc)^{\frac{1}{2}} + +P6(mc)^{\frac{1}{2}} + P7(imc)^{\frac{1}{2}} + P8(mc)^2 + P9(imc)^2$	0.93	0.086	(30)
	$k = P1 + P2(pc) + P3(mc) + P4(imc) + P5(pc)^{\frac{1}{2}} + P6(mc)^{\frac{1}{2}} + P7(imc)^{\frac{1}{2}} + P8(mc)^2 + P9(imc)^2 + P10(mc)(pc) + P11(mc)^{\frac{1}{2}}(pc)^{\frac{1}{2}}$	0.94	0.090	(31)
Non-Linear:	$Lnk = [pc \cdot imc/(P + P2 \cdot imc)] \cdot [P3 + P4 \cdot mc]$	-	0.050	(32)
	$Lnk = [P1 + P2 \cdot mc + mc \cdot imc/(P3 + P4 \cdot imc)] \cdot [P5 + P6(pc)^{\frac{1}{2}}]$	-	0.015	(33)*
	$Lnk = [pc/P1 + P2 \cdot pc + pc \cdot mc/P3 + P4 \cdot pc] \cdot [P5 \cdot imc + P6(imc)^2]$	-	0.046	(34)

*Values of Constants for Selected Model (8):

$$Lnk = [P1 + P2 \cdot mc + mc \cdot imc/(P3 + P4 \cdot imc)] \cdot [P5 + P6(pc)^{\frac{1}{2}}]$$

Constant	Value	Standard Deviation
P1	1.607	0.56
P2	-1.611	0.57
P3	0.034	0.03
P4	0.309	0.00
P5	1.520	0.53
P6	-0.482	0.02

In Eq. 33, the introduction of a protein effect on the rate constant into the combination effects of moisture content and initial methionine concentration improved the model, implying a close relationship between protein content and the other two variables. The estimated constants for this equation, the mathematical model (Model 8) considered the most suitable, are presented in Table 3.

Development of an Overall Kinetic Model. In order to combine the temperature effect on methionine degradation with the model for the other three independent variables considered, it was necessary to observe the interactions between temperature and moisture content and/or protein content. Observation of the activation energy as influenced by moisture content showed that at high

TABLE 4.
RATE CONSTANTS (k) IN INCOMPLETE BLOCK EXPERIMENTAL DESIGN

Temperature (T) (°K)	Moisture Content (%)	Initial Meth. Conc. (%)	Protein Content (%)		
			20	40	60
363	10	0.5	-	0.7	-
	20	0.5	0.4	0.4	0.4
	30	0.5	-	0.2	-
383	10	0.5	-	1.6	-
	20	0.5	1.8	1.7	1.6
	30	0.5	-	0.8	-
403	10	0.5	-	4.1	-
	20	0.5	4.5	3.7	4.1
	30	0.5	-	3.4	-
413	10	0.3	6.7	5.4	6.0
		0.5	7.3	7.0	6.3
		0.7	6.0	5.8	5.1
413	15	0.3	5.3	4.8	4.2
		0.5	6.1	6.0	5.1
		0.7	4.9	4.8	4.0
413	20	0.3	5.1	4.5	4.8
		0.5	5.8	5.8	4.5
		0.7	4.8	5.1	4.3
413	30	0.3	4.2	3.9	2.7
		0.5	4.8	4.8	3.9
		0.7	3.8	4.0	3.4

TABLE 5.
OVERALL KINETIC MODELS FOR METHIONINE DEGRADATION

Trial Models	s^2	Equation No.
$Lnk = P1 + P2/T + P3/T^2 + [P4 \cdot mc/T + mc \cdot imc/T^3(P5 + P6 \cdot imc)] \cdot [P7 + P8(pc)^{\frac{1}{2}}/T]$	0.023	(35)
$Lnk = P1 + P2 \cdot T + P3/T + [mc \cdot imc/T^3(P4 \cdot imc + P5) + P6 \cdot mc/T] \cdot [P7 + P8(pc)^{\frac{1}{2}}/T]$	0.024	(36)
$Lnk = P1 + P2 \cdot T + P3/T + [mc \cdot imc/(P4 + P5 \cdot imc) + P6 \cdot mc/T^2] \cdot [P7 + P8(pc)^{\frac{1}{2}}]$	0.021	(37)*
$Lnk = P1 + P2 \cdot T + [mc \cdot imc/(P3 \cdot imc + P4) + P5 \cdot mc] \cdot [P6 + P7(pc)^{\frac{1}{2}}/T]$	0.032	(38)
$Lnk = P1 + P2 \cdot T + [mc \cdot imc/T(P3 \cdot imc + P4) + P5 \cdot mc] \cdot [P6 + P7(pc)]$	0.025	(39)

*Values of Constants for Selected Model (9):

$$Lnk = P1 + P2 \cdot T + P3/T + [mc \cdot imc/(P4 + P5 \cdot imc) + P6 \cdot mc/T^2] \cdot [P7 + P8(pc)^{\frac{1}{2}}]$$

Constant	Value	Standard Deviation
P1	1.3938×10^2	3.38
P2	-0.1537	0.04
P3	-3.0495×10^4	6.51
P4	4.5848×10^3	6.55
P5	-5.8895×10^5	48.86
P6	-1.8602×10^6	0.00
P7	0.0751	0.11
P8	0.2386	0.82

moisture levels, the systems had slightly higher activation energies. However, a relationship between temperature and protein content was not observed.

An overall kinetic model was generated by adding the temperature effect, as described by the Arrhenius equation, to the model of three independent variables. Incomplete block designs were conducted to examine the interactions between the temperature and moisture content (Table 4). Based on experimental data and the models for three independent variables, the overall temperature effect on methionine degradation was established by nonlinear least squares fitting (Table 5). Equation (37) showed the least average s^2, generating eight constants. Therefore, this overall model as a function of four independent parameters affecting methionine degradation was selected as describing methionine degradation kinetics. Estimated values for the constants of this model (Model 9) are given in Table 5.

CONCLUSIONS

An overall mathematical model describing the kinetics of degradation of methionine was developed based on experimental observations obtained at isothermal conditions. For the building of such a model, the partial responses of methionine degradation as a function of major parameters such as temperature, moisture content, protein content and initial methionine concentration were combined. The influence of several parameters operating simultaneously was monitored in an effort to establish their interdependence as affecting the methionine degradation reaction. In general, good correlations between predicting models and experimental data were observed indicating that it is feasible to mathematically describe the losses of methionine. Such models can be subsequently used in trying to establish optimum processing conditions when information on composition of the system and heat and mass transfer is available.

Mathematical models developed for these soy-based systems may apply to other soy systems undergoing nonenzymatic browning reactions. Furthermore, it is possible that by extrapolation, these overall models can be used to forecast the storage stability of methionine-containing systems.

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PREDICTION OF METHIONINE RETENTION DURING EXTRUSION PROCESSING OF FORTIFIED SOYBEAN MODEL SYSTEMS

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ABSTRACT

The effect of extrusion on the nutritional quality of processed products is vastly unknown. This investigation has explored the possibility of predicting methionine losses in fortified soy-model systems using a twin-screw extruder. Since the rate of degradation of methionine has been found to be greatly affected by temperature, mathematical models to describe the extrusion behavior of a system in which sample temperature varies according to location within the extruder and process temperature were established. In this investigation, heat transfer through the barrel wall and frictional heat generated from the rotation of the screws were considered the major factors in determining the actual temperature of the sample. In order to calculate methionine retention during extrusion, it was highly important to determine residence time distribution. The time required for the material to travel through the individual zones of the extruder was of critical importance to accurately predict methionine retention. Mathematical models describing the kinetics of methionine degradation as a function of all parameters and mathematical models describing extrusion behavior were combined to predict the retention of methionine. The simulation was verified by comparing predicted values with experimental data. It was found that the calculated values from this simulation presented the same trends for methionine degradation as those of experimental data with regard to moisture content, protein content, initial methionine concentration and temperature. In general, a reasonable agreement between predicted and experimental values was obtained.

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INTRODUCTION

Food extrusion has become an important processing technique applied to many raw materials and protein sources such as soybeans, peanuts and cottonseed (Altschul and Wilcke 1985). The advantages of using extrusion cooking have been evaluated by Smith (1976) and Harper (1978). Extruders can achieve high productivity and can handle a wide variety of raw ingredients and processing conditions. The high-temperature processing capability of extruders with short residence times, termed HTST processing, minimizes nutrient degradation while destroying most microorganisms. Nevertheless, extruders may also extensively affect food product quality, specifically, vitamin content, enzyme activity and amino acid availability in foods, depending on processing conditions.

These undesirable changes in heat sensitive materials may be minimized by the application of twin-screw extrusion. Intermeshing twin-screw extruders with self-wiping ability would produce high rates of heat transfer, hence, minimize processing time. At present, however, twin-screw extruders have not been extensively used in food processing, and most studies reported have employed single-screw extrusion technology (Gordon 1969, Smith 1976).

Although limited work for twin-screw extruders has been carried out for its adaptation to food processes, twin-screw extrusion processing of soy and other plant flours, concentrates and protein isolates holds a very promising future due to its flexibility and advantages. Sander (1982) utilized twin-screw extrusion in processing soy flours with as low as 20% nitrogen solubility and reported successful texturization.

Literature information on the effect of extrusion on the nutritional quality of food materials is very limited. The degradation of lysine due to the Maillard reaction was investigated by Cheftel *et al.* (1981) during the extrusion cooking of protein-enriched biscuits in a co-rotating twin-screw extruder. Aguilera and Kosikowski (1976) examined the relationship between extrusion conditions and product characteristics as a means of optimizing the process through simultaneous analysis of temperature, feed moisture content and screw speed.

Since the temperature of the sample varies according to its position within the extruder and temperature of the process, a mathematical model describing temperature of the sample as a function of these effects needs to be developed. Most studies for temperature profiles have been done to calculate sterilization values in conduction-heated canned foods (Lenz and Lund 1980; Stumbo 1973; Teixeira *et al.* 1969). Villota and Karel (1980a,b) developed a mathematical model from experimental data to describe the temperature profile of a model system during dehydration as a function of time at any dry-bulb temperature. In extrusion, Holay and Harper (1982) estimated the dough temperature profile from the temperature information of the dough and barrel. For the specific case of twin-

screw extruders, limited work has been reported to describe temperature profiles. Stasiek (1974) and Klenk (1971) provided only some idea of the complexity expected in twin-screw extruders.

Special techniques have been developed to predict the shelf-life of packaged stored foods on the basis of laboratory tests designed to establish the kinetics of deterioration and mass transfer properties of packaging materials (Karel 1975 and Labuza 1973). Although a number of studies has been carried out on the prediction of storage stability (Riemer and Karel 1978; Quast and Karel 1972; Simon *et al.* 1971), the literature is very scarce in studies that would predict the extent of nutrient deterioration during processing. Villota and Karel (1980a,b) developed a methodology for the prediction of ascorbic acid retention during an air-drying process. Mathematical models to describe the drying behavior of a system were developed by establishing moisture and temperature distributions in the sample as a function of time. Mathematical models for kinetics of degradation and dehydration behavior were combined to predict retention as a function of time and drying conditions.

Therefore, it is the goal of this investigation to study the feasibility of predicting methionine retention in an extrusion process by developing kinetic models and analyzing heat transfer behavior within a twin-screw extruder.

MATERIALS AND METHODS

Raw Materials

Model systems (40 60% soy protein isolates, 0.3 0.7% methionine, 4% D-glucose and a balance of microcrystalline cellulose to make 100% solids) were finely milled and properly mixed prior to extrusion to allow more intimate and rapid mixing of the various ingredients. Dry ingredients were mixed for 10 min by using a Blakeslee ball mixer (G. S. Blaskeslee Co., Chicago, IL). The solid raw materials were then fed into the extruder's hopper using a volumetric screw feeder.

Equipment

A Werner Pfleiderer ZSK-30 twin-screw extruder (Werner Pfleiderer Corp., Ramsey, NJ) with co-rotating intermeshing screws was utilized in our experiments.

Feeding. Raw materials were introduced into the feed zone by means of a K-Tron Model T35 twin-screw volumetric feeder (K-Tron Corp., Glassboro, NJ) as a single premix stream, and flow rates regulated by a K-Tron Series 6300 digital speed controller (K-Tron Corp., Glassboro, NJ). Water was added into

the feed zone with a Masterflex® pump (Cole-Parmer Instrument Co., Chicago, IL) to adjust moisture content of the material undergoing extrusion.

Screw Barrel. The screw barrel of the extruder consisted of individual barrel sections held together by tie-rods and supported on the machine frame. The number of barrel sections may also be varied to obtain different processing lengths ranging from 15 to 36 L/D (L = processing length, D = diameter of screw) as required, depending on the application. For the extrusion experiments in this investigation, a processing length of 36L/D was used and sampling ports were selected at three different locations, namely 17L/D, 23L/D and 29L/D. Each section of the extruder was electrically heated and cooled by water. The barrel temperature was regulated by a temperature controller (Barber-Coleman Co., Lovespark, IL) at five different points along the length of the extruder.

Screws. The screws consisted of continuous shafts in which screw flighted components and special kneading elements were installed, according to a predetermined profile in order to achieve an appropriate degree of texturization. The specific configuration of the screws used in this study is presented in Fig. 1.

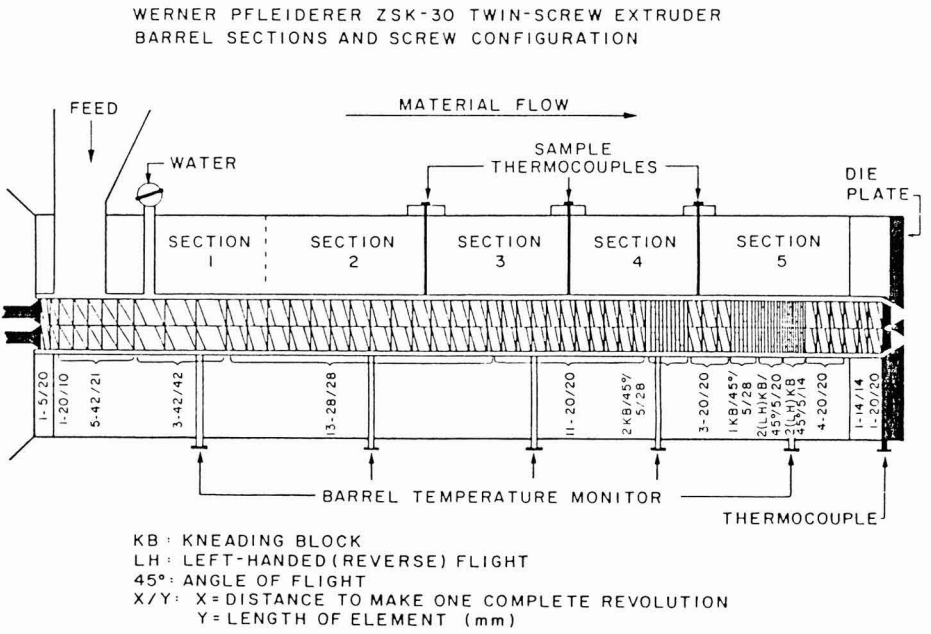

FIG. 1. SCHEMATIC DIAGRAM OF A WERNER PFLEIDERER ZSK-30 TWIN-SCREW EXTRUDER VIEWING BARREL SECTIONS AND SCREW CONFIGURATION

The particular design of the screws made it possible to include successive stages namely a conveying, a mixing and finally a texturization section. Appropriate screw components were used to draw the feed material into the barrel and convey it forward; additional shear during extrusion was obtained by including a number of kneading blocks.

Die Configuration. The die for this extrusion system was a single plate with two round openings (5 mm in diameter).

Experimental Procedure

Measurement of Sample Temperature. Sample temperatures at different locations were measured to establish a complete temperature profile which is necessary for the development of heat transfer models. The temperature of the dough in the extruder was measured with TEX Probes (Omega Eng. Inc., Stanford, CT) at three locations and at the die of the extruder with a Mico 873 indicator (Mele Instrument Co., Marlboro, NJ). Thermocouples were placed in the vent ports of the barrel section (Fig. 1) to measure the dough temperature which was monitored by means of a temperature potentiometer (Leeds and Northrup Co.)

Measurement of Retention Time. Total retention time was measured by injecting dyes and measuring the time required for the material to appear at the die. Two different powdered dyes were used: FD&C Aluminum Lake (Blue #2, Colorcon, Inc.) and Sudan III (Fisher Scientific Co.). A few milligrams of dye were dropped at the feed hopper, and the holding time in the extruder was measured. To determine the residence time distribution within the extruder, a small amount of dye was introduced at the feed hopper, and the time required to travel from section to section was measured. The residence time of the dye was determined for three individual sections as well as for the overall unit at constant screw speed (300 rpm).

Methionine Determination during Extrusion. Product obtained by direct expansion at the extruder die was collected and final levels of methionine measured. To examine methionine retention during the process, samples were collected through a right angle pipe by opening the sampling ports in the last three consecutive sections.

A fraction of each sample was then placed in a vacuum oven at 62°C for 24 h. to measure moisture content. The remaining sample was ground to facilitate methionine extraction. Exactly 500 mg of the sample were weighed and extracted with 7 mL of 10% methanol in water. Three milliliters of the extract, 0.5 mL of phosphate buffer and 1 mL of dansyl chloride solution were mixed and heated for one hour at 40°C. Dansyl derivatives of methionine were then analyzed by HPLC (Choi and Villota 1989).

RESULTS AND DISCUSSION

Temperature Profiles

Methionine degradation rates have been previously determined to be strongly affected by temperature (Choi and Villota 1989). Since temperature changes with time and location within the extruder, a mathematical model describing temperature of the sample as a function of process temperature at any given location was found necessary in heat transfer analyses.

The screw barrel of this unit is made up of five individual barrel sections (Fig. 1). The temperature of each section was monitored with a temperature controller, and the actual barrel temperature recorded. Two heat sources may be important during extrusion: electrical heat supplied from the barrel at a given processing temperature and frictional heat generated by rotation of the screws; therefore, sample temperature was different from barrel temperature.

Actual barrel temperatures and sample temperatures were measured for nine different sets of processing temperatures in samples containing several moisture content levels (Table 1), keeping processing temperatures in the first and second sections constant. In general, high temperatures in the feed zone have been found to be undesirable. Thus, temperatures of the first two sections were kept at 50° and 100°C for the first and second sections, respectively. Temperature of the sample in the third section seemed to be a function of only the processing temperature of section 3 with constant processing temperatures in the first and second sections.

In sections 4 and 5, however, temperature of the sample was not only a function of temperature of the process but also a function of temperature of the sample in the previous section. The frictional heat generated by shear may significantly affect the sample temperature, depending on the viscosity of the material. Viscous heating is most significant in high viscosity materials; however, viscosity is in general considered to be relatively temperature dependent (Remsen and Clark 1978; Jao and Chen 1978; Clark 1978). Therefore, the effect of temperature on frictional energy should be introduced in models describing the distribution of sample temperature during the extrusion process. The temperature of the sample was also measured at the die. In this last zone, the extruder screws have kneading blocks which create highly vigorous agitation and provide high shear mixing; therefore, the effect of viscous dissipation is very important.

Another important factor is the location of thermocouples to monitor sample temperature. Thermocouples were placed between two barrel temperature controllers; therefore, the temperature of the sample as measured by the thermocouples was affected by two processing temperatures.

In section 3, the temperature of the sample was lower than the barrel temperature due to heat transfer through the barrel wall near the feed zone. However,

TABLE 1.
TEMPERATURE PROFILES DURING EXTRUSION.
SECTION 1 = 50°C SECTION 2 = 100°C

Sample No. 1:		Barrel Temperature			Sample Temperature		
Section No.	Processing Temperature \ Moisture Content:	40%	36.5%	32%	40%	36.5%	32%
3	120	116	115	117	107	106	108
4	150	144	147	146	145	148	150
5	140	137	140	140	142	146	148
[a] PT:					140	143	147

[a] PT: Product temperature at the die

Sample No. 2:		Barrel Temperature			Sample Temperature		
Section No.	Processing Temperature \ Moisture Content:	40%	36.5%	35%	40%	36.5%	35%
3	120	117	120	122	105	106	106
4	160	159	159	157	162	160	163
5	150	152	151	150	154	157	156
PT:					142	145	152

Sample No. 3:		Barrel Temperature	Sample Temperature
Section No.	Processing Temperature[b] \ Moisture Content:	40%	40%
3	120	116	104
4	170	161	159
5	160	156	154
PT:			150

[b] This temperature profile required a minimum of 40% moisture content in the system.

TABLE 1.
(CONTINUED)

Sample No. 4:		Barrel Temperature			Sample Temperature		
Section No.	Processing Temperature \ Moisture Content:	40%	36.5%	32%	40%	36.5%	32%
3	130	113	119	120	114	113	115
4	150	135	148	155	147	150	157
5	140	136	142	147	144	148	154
PT:					135	143	148

Sample No. 5:		Barrel Temperature			Sample Temperature		
Section No.	Processing Temperature \ Moisture Content:	40%	36.5%	32%	40%	36.5%	32%
3	130	130	130	132	113	115	115
4	160	158	157	158	164	165	165
5	140	139	141	142	155	155	156
PT:					136	140	142

Sample No. 6:		Barrel Temperature			Sample Temperature		
Section No.	Processing Temperature \ Moisture Content:	40%	36.5%	32%	40%	36.5%	35%
3	130	132	131	132	118	117	116
4	170	160	161	161	166	165	166
5	150	153	149	150	158	159	160
PT:					148	145	151

TABLE 1.
(CONTINUED)

Sample No. 7:		Barrel Temperature			Sample Temperature		
Section No.	Processing Temperature / Moisture Content:	40%	36.5%	32%	40%	36.5%	32%
3	140	134	135	137	117	118	118
4	170	164	159	158	166	168	169
5	160	153	151	150	157	157	159
PT:					150	149	157

Sample No. 8:		Barrel Temperature	Sample Temperature
Section No.	Processing Temperature / Moisture Content:	40%	40%
3	140	136	118
4	180	167	172
5	160	163	162
PT:			159

Sample No. 9:		Barrel Temperature			Sample Temperature		
Section No.	Processing Temperature / Moisture Content:	40%	36.5%	32%	40%	36.5%	35%
3	150	138	139	140	119	120	121
4	150	152	152	152	168	172	173
5	150	149	148	149	155	158	161
PT:					145	151	155

Table 1 shows that temperatures of the sample in the two sections near the die were higher than the barrel temperatures. It is expected that the temperature of the material near the feed zone is mostly determined by the heat transfer through the barrel wall. On the other hand, materials conveyed to the transition zone are mixed, compressed and delivered to the die. In this zone, the viscous dissipation effect may be considered significant. Therefore, the temperature of the sample generally continues to increase due to the generation of frictional energy in the metering zone of the extruder.

A model describing sample temperature was established in each section by combining the effect of viscous dissipation and heat transfer at the barrel wall. In this simulation, the first section was neglected due to the assumption that there was no appreciable methionine degradation due to the low temperatures.

The dependent variable which is the temperature of the sample (TS) was studied as a function of processing temperature (TP) and/or the sample temperature of the previous section (TSi, i = barrel section No.). The approach taken to develop these models was the one that considers that a functional relationship can be generated by fitting appropriate functions to a response. For this purpose, a linear program of the Statistical Analysis System (SAS) was used. As far as nonlinear models were concerned, it was concluded that no further improvement of the mathematical models could be achieved by using this approach. In this study, a model system containing 40% protein, 0.5% methionine and 36.5% water was extruded at 300 rpm.

Development of a Model for Section 2. A model for the temperature profile in section 2 was established as a function of processing temperature of section 3, based on the heat transfer at the barrel wall. The material entered the feed zone and was conveyed to the next section without cooking; in this section, therefore, the frictional heat may be considered negligible. Equation (1) was established with a significant F value of 40.4 and r^2 of 0.99 (Table 2). The actual values of the coefficients (P) and the standard errors are also presented.

Development of a Model for Section 3. In the third section, the material may be compacted and converted from a flowing granular or sticky mass to a relatively uniform plasticized dough. Therefore, viscous dissipation heat should be considered as an influential component with respect to an increase in sample temperature in this section. It is generally recognized that frictional energy is critical in modeling temperature profiles in the cooking zone. Viscosity of the dough has a great deal of influence on the frictional heat and is a function of the extrusion temperature. The effect of temperature on viscosity was represented by an Arrhenius equation for a Newtonian fluid (Glasstone *et al.* 1941) and for pseudoplastic materials (Metzner 1967). Based on this study, Eq. (2) was established as a function of processing temperature of the third section (TP3), processing temperature of the fourth section (TP4) and the sample temperature of the second section (TS2) as shown in Table 2.

TABLE 2.
DEVELOPMENT OF MODELS WITH 36.5% MOISTURE CONTENT FOR DIFFERENT BARREL SECTIONS WITHIN THE EXTRUDER

1. Model for Section 2:
$TS2 = P1 + P2\ (TP3) + P3\ [Ln\ (TP3)]$ Eq. (1)

Coefficient	Value	Standard error
P1	-2095.458	87.54
P2	-3.613	0.16
P3	550.444	2.24

2. Model for Section 3:
$TS3 = P1 + P2\ (TP3) + P3\ Ln\ (TP3) + P4\ [Ln\ (TP4)] + P5\ (TS2) + P6\ [EXP\ (1/TS2)]$ (Eq. (2)

Coefficient	Value	Standard error
P1	-1.109×10^6	438.20
P2	-8.794	0.94
P3	1540.672	56.12
P4	-548.713	28.12
P5	94.888	9.83
P6	1.085×10^6	431.73

3. Model for Section 4:
$TS4 = P1 + P2\ (TP4) + P3\ Ln\ (TP4) + P4\ [Ln\ (TP5)] + P5\ (TS3) + P6\ [EXP\ (1/TS3)]$ (Eq. (3)

Coefficient	Value	Standard error
P1	-1.618×10^4	383.06
P2	-6.167	0.33
P3	938.342	49.03
P4	7.939	1.20
P5	0.986	0.14
P6	1.229×10^4	370.80

TABLE 2.
(CONTINUED)

4. Model for Section 5 (at the die):
TS5 = P1 + P2 (TP5) + P3 [Ln (TP5)] + P4 (TS4) + P5 [EXP (1/TS4)] (Eq. (4)

Coefficient	Value	Standard error
P1	-1.598×10^4	807.92
P2	-8.735	1.74
P3	1433.440	56.86
P4	6.391	0.92
P5	1.521×10^4	792.54

The last two terms of Eq. (2) indicate the viscous dissipation heat, while the P1, P2 and P3 terms represent heat transfer through the barrel wall. Although this model exhibited some discrepancy between experimental data and predicted values as shown by the relatively low value of $r^2 = 0.96$, an acceptable fit was obtained with an F value of 34.6. The coefficients P1, P2, P3, P4, P5 and P6 were determined through computer calculations (Table 2).

Development of a Model for Section 4. The heat transfer behavior in this section may be assumed to be very similar to that in section 3. It is, however, possible that the effects of frictional heat may be more significant in this section due to the presence of kneading blocks. Therefore, the best fit was obtained by the same model as that in section 3 (Eq. 2) but with different values of the coefficients (Eq. 3, Table 2) and with $r^2 = 0.98$, and $F = 34.2$.

Development of a Model at the Die. Because the chambers of the extruder's screw are completely filled with material near the discharge end, a significant amount of viscous dissipation of the mechanical energy used to turn the screws occurs in these chambers with correspondingly high shear rates. Since one side of the die plate is exposed to ambient temperature, assumed constant, a modification to the model for the previous section was necessary to find the best correlation to describe temperature of the sample at the die. This section included both heat transfer through the barrel wall and frictional heat from viscous dissipation. If the ambient temperature can be assumed constant, the model may be established as a function of the processing temperature (TP5) in section 5

and the sample temperature in section 4 (TS4), as shown in Equation 4 (Table 2). The r^2 was calculated to be 0.98 with F = 49.4. Results indicate that the model describes experimental data quite well with five constants (Table 2).

In summary, temperature profiles in twin-screw extruders are absolutely necessary for the development of heat transfer models which in turn are required to determine the behavior of a system undergoing extrusion. Unfortunately, it is extremely complex to establish a detailed temperature description due to radial and longitudinal temperature gradients, reactivity of material and associated viscosity changes and partial fill in some of the C-chambers.

Temperature profiles were established for this study as a function of processing and sample temperatures keeping other parameters constant to remove complexity. When temperature of the sample was also investigated for different moisture content systems, it appeared to decrease as moisture content increased due to less frictional heat generated from viscous dissipation. Mechanisms affecting sample temperature may not change, but the magnitude of each effect will depend on the amount of water added. Therefore, the estimates of coefficients for models describing the sample temperature should be determined at each moisture content, although the same equations are valid.

Several factors may have contributed to cumulative errors which would lead to differences between experimental and predicted values. A factor that perhaps is of primary importance is flow patterns. In general, only the chambers at the die end are expected to be fully filled with material while the rest may be partially empty. Therefore, the effect of heat transfer and/or viscous dissipation heat on the temperature of the sample may change. Another possible source of error is the assumption of the same sample temperature in the radial direction due to the good mixing capabilities of twin-screw extruders. Measurements of Klenk (1971) indicated the complexity of the radial-temperature distribution in twin-screw extruders. However, it should be stressed that because of the intermeshing characteristics of the screws in our twin-screw extruder, it is expected that material with very high uniformity will be present in each individual chamber.

Moisture Profiles in the Extruder

The effect of moisture level is critical not only on temperature profiles but also on the rate constants of methionine degradation. However, it can be safely assumed that there was no loss of moisture during the extrusion process per se and that the moisture level changed only as the material left the die due to the design of the extruder since venting was not included in this process. Therefore, overall development of mathematical models for extrusion behavior was performed with the assumption that there is no moisture loss during the extrusion process.

Residence Time Distribution

In dealing with systems in which a time-dependent reaction is monitored, it is critical to determine the time required for the material to travel through the extruder. Since there are several types of leakage flows within a twin-screw extruder, it is possible that not all the material is extruded uniformly and that some may remain longer in the extruder. It was also found that residence times in the individual sections were different due to different configurations of the screw. Therefore, the residence time of the material in each section was determined by observing both time of appearance and disappearance of the dye (Table 3). Individual residence times in each section were also calculated from Table 3 and reported in Table 4.

TABLE 3.
ARRIVAL TIME FROM THE FEED HOPPER TO EACH SECTION

Section	Appearance time (sec)	Disappearance time (sec)
2	5-6	9-11
3	9-10	18-23
4	14-16	30-32
5*	33-34	54-56

*indicates the time that the material appeared at the die

TABLE 4.
RESIDENCE TIME IN INDIVIDUAL SECTION

Section	SRT[a] (sec)	LRT[b] (sec)
1 & 2	5-6	9-11
3	3-5	9-14
4	4-7	7-14
5	17-20	22-26

[a] SRT: shortest residence time for materials in each section

[b] LRT: longest residence time for materials in each section

Results suggest that the last zone may generate high back mixing showing long residence times, most likely due to the effective action of reverse-flight screw elements. Furthermore, temperatures generally continued to increase in the final or metering zone. Therefore, the final zone was considered to be of critical importance to methionine degradation.

Simulation

Mathematical models describing the kinetics of methionine degradation (Choi and Villota 1989) and extrusion behavior (temperature profiles of the sample and residence time distributions) were combined to determine the retention of methionine during extrusion through simulation carried out by a computer according to the flow chart presented in Fig. 2.

A new reaction rate for methionine degradation was determined according to the temperature profile of the sample in every section along the length of the extruder. Total loss of methionine was calculated by summation of the partial losses of methionine in each section. A factor that needs to be examined is the problem of clearly establishing an accurate temperature profile within a section because of the limited number of temperature sensors available (Fig. 1). The temperature of the sample is expected to change progressively along each individual section, increasing or decreasing according to the temperatures of the neighboring zones. Therefore, it was assumed that the sample temperature increased or decreased uniformly and linearly between two consecutive temperature-monitored points. This assumption may be reasonable since mathematical models of temperature profiles (Eq. 1 through Eq. 4) suggest a linear relationship between sample and processing temperatures. A FORTRAN computer program was developed to predict methionine retention during extrusion and computation was carried out in a CYBER 175 system.

Verification

Verification was required to determine whether or not the mathematical expressions adequately represented the response of the real systems.

Measurements of Methionine Retention in Each Section. Verification was conducted for each section of the extruder to examine the "goodness" or "badness" of the predictions. Samples were collected at the end of each section and at the die, and the remaining methionine was measured and compared with expected values (Fig. 3). The predicted values were estimated using two different residence time distributions. Actual data showed that there is no degradation of methionine up to the second section, possibly because of the uneven distribution of moisture, low shear, and moderately low process temperatures. In the last two sections, however, experimental data indicated less methionine retention

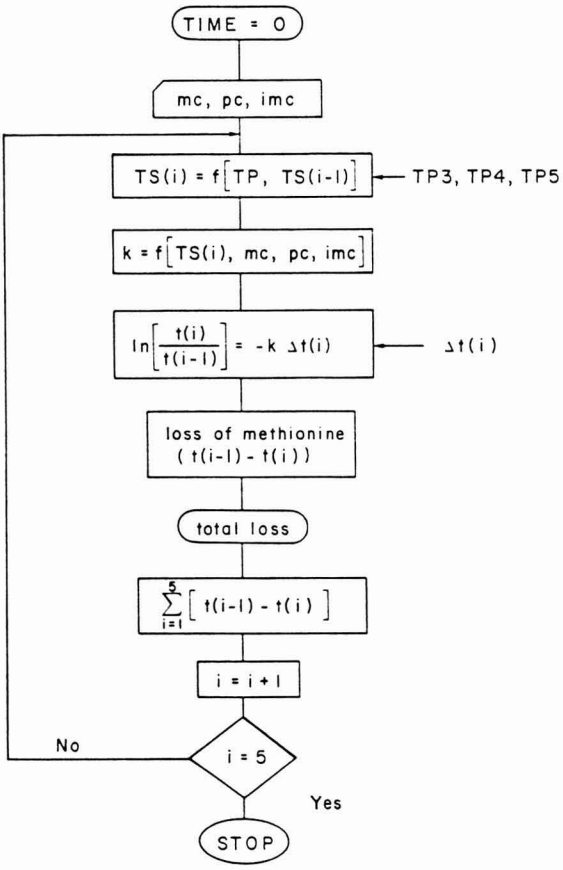

FIG. 2. GENERAL FLOW DIAGRAM FOR THE PREDICTION OF METHIONINE RETENTION DURING EXTRUSION

$\Delta t(i)$ = time interval according to residence time of the material in each section.
i = section number
imc = initial methionine concentration
mc = moisture content
pc = protein content

than predicted values. The effect of shear rate may tend to increase the breakdown of methionine, since increased turbulence may facilitate the interaction between methionine and other components in the system. Kinetic models, however, were not developed taking this parameter into account. Furthermore, the great reduction in moisture content due to puffing in the last zone may affect methionine degradation.

Although samples were collected immediately upon emerging from the die following flash vaporization, it is possible that additional methionine may be

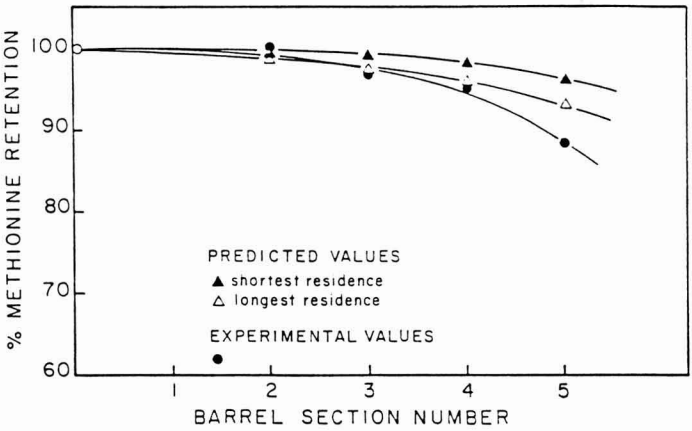

FIG. 3. THEORETICAL VERSUS EXPERIMENTAL VALUES FOR METHIONINE DEGRADATION DURING EXTRUSION AS A FUNCTION OF BARREL SECTION (MODEL SYSTEM: 40% PROTEIN/4% D-GLUCOSE/0.5% INITIAL METHIONINE CONTENT)
Dry feed rate: 35 lb/h; water feed rate: 20.1 lb/h; screw speed: 300 rpm

degraded after expansion due to the high temperature within the sample. Relatively high moisture existed in the extrudate as evidenced by samples extruded at an initial moisture content of 36.5% which exhibited a final moisture of 26%.

Effect of Moisture Content. Although samples with two different moisture contents (36.5% and 40%) presented two similar temperature profiles, different values for the coefficients were estimated due to differences in the magnitude of frictional energy. In order to examine the deviation between experimental data and predicted values, samples containing two different moisture contents were extruded at several combinations of the barrel temperatures at 300 rpm. Temperatures, however, for sections 1 and 2 were maintained at 50° and 100°C, respectively. Results are given in Table 5. Major factors for the discrepancies in methionine retention may include the effect of shear rate, the moisture drop at the die, minor temperature gradients in the radial direction and approximation of the temperature profiles in each zone. However, deviations of the predicted values from experimental data were reasonably uniform over the whole range of temperatures, showing a 5 to 7% difference.

Effect of Temperature. It was observed through the course of this investigation that the rate of methionine degradation increased as temperature increased following an Arrhenius correlation. Temperature of the sample should be considered and calculated by the models describing temperature profiles inside the barrel during extrusion in order to accurately estimate methionine degradation. Nine sets of experiments with different combinations of barrel temperatures were

TABLE 5.
COMPARISON OF PREDICTED AND EXPERIMENTAL VALUES FOR METHIONINE DEGRADATION

Experiment	Barrel Temperature Section 3	Section 4	Section 5	Methionine Retention (%) 36.5% moisture content Experimental	Predicted	40% moisture content Experimental	Predicted
1	130	150	140	89.0	92.8[a]-96.1[b]	82.3	94.1[a]-97.2[b]
2	150	150	150	87.2	91.0-93.8	91.2	92.2-95.4
3	120	160	150	87.5	91.8-94.9	91.2	93.2-96.3
4	120	150	140	90.0	93.0-96.4	93.0	94.3-97.4
5	130	170	150	87.8	91.2-94.0	89.9	92.3-95.5
6	130	160	140	89.8	92.0-95.3	92.0	93.2-96.5
7	120	170	160	--	90.5-93.4	87.7	91.9-95.0
8	140	170	160	80.2	89.8-92.5	87.5	90.5-93.4
9	140	180	160	80.0	87.2-90.6	85.1	89.4-92.2

[a] = Predicted values with longest residence time
[b] = Predicted values with shortest residence time

conducted to examine the deviation between experimental data and predicted values (Table 5). Although there was some disagreement, the effect of temperature on methionine degradation seemed to be described reasonably well by the Arrhenius equation, showing a similar trend between predicted and experimental values (Choi and Villota 1989).

Effect of Protein Content. Kinetic studies demonstrated that the addition of protein showed a catalytic-like effect on the degradation of methionine for concentrations up to 25%. However, any increase in protein above 25% reduced the reaction rate (Choi and Villota 1989). Hence, a kinetic model for the degradation of methionine was evaluated as a function of protein content. Samples with two different protein contents (40% and 60%) were extruded at 300 rpm. Barrel temperatures were adjusted to 50°C in Section 1, 100°C in Section 2, 130°C in Section 3, 150°C in Section 4 and 140°C in Section 5. It was shown that methionine retention increased as protein content increased according to our experimental data and predicted values (Table 6).

Effect of Initial Methionine Content. In our kinetic studies, it was observed that methionine degradation rates were greatly influenced by the initial concentration of methionine (Choi and Villota 1989). The rate of methionine degradation increased as the concentration of methionine increased from 0.3 to 0.5%. However, the rate of methionine degradation after passing through a maximum at 0.5% decreased again as concentration of methionine increased to 0.7%. This trend was also observed in samples containing three different methionine concentrations and extruded with the following processing temperatures: Section 1, 50°C; Section 2, 100°C; Section 3, 130°C; Section 4, 150°C; and Section 5, 140°C.

The effect of initial methionine concentration seemed to be less marked in the extrusion process (Fig. 4) than in kinetic studies (Choi and Villota 1989). A possible explanation for the lower retentions observed during extrusion might

TABLE 6.
COMPARISON BETWEEN PREDICTED AND EXPERIMENTAL VALUES FOR METHIONINE RETENTION

	Methionine Retention (%)	
Protein Content	Actual	Predicted
40	89.0	92.8[a]-96.1[b]
60	90.2	93.4[a]-96.3[b]

[a] Predicted values with longest residence time
[b] Predicted values with shortest residence time

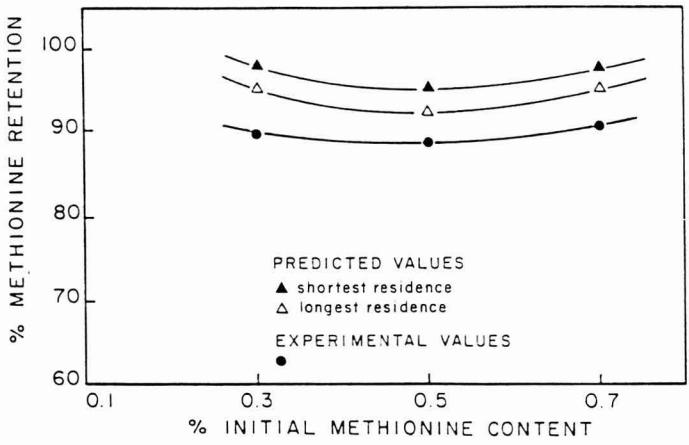

FIG. 4. THEORETICAL VERSUS EXPERIMENTAL VALUES FOR THE EFFECT OF INITIAL METHIONINE CONCENTRATION ON METHIONINE RETENTION DURING EXTRUSION (MODEL SYSTEM: 40% PROTEIN/4% D-GLUCOSE CONTENT)
Dry feed rate: 35 lb/h; water feed rate: 20.1 lb/h; screw speed: 300 rpm

be the generation of high shear rates due to strong mixing during extrusion which would increase the availability of methionine independent of its concentration.

CONCLUSIONS

Our results show that simulation by combining mathematical models describing the kinetics of methionine degradation and extrusion behavior can predict the effect of major parameters reasonably well. The simulation satisfactorily represented the trends of methionine degradation with regard to moisture content, temperature, protein content and initial methionine concentration. Several factors may have contributed to the discrepancies observed between experimental and predicted values. One of the main reasons for these differences may be the significant moisture loss at the die. In fact, when extrusion was simulated before the point of expansion, the accuracy of simulation was much higher. The denaturation of protein due to shear rate during extrusion may have also contributed to some degree of inaccuracy. The degree of protein denaturation occurring during extrusion will vary in each section which in turn may result in a different degree of interaction between protein and glucose. Other factors may play a minor role in causing differences between experimental and predicted values such as shear rate, flow patterns, pressure fluctuation, melting mechanisms, etc.

In general, it is considered that, regardless of the complexity encountered in extrusion processes, simulation and prediction can be successfully accomplished. However, a better knowledge of the extrusion behavior will improve the accuracy

of mathematical models describing methionine degradation during extrusion. In our simulation studies, moisture loss of samples at the die, shear rate and flow patterns during extrusion are considered to be among the most important sources of error and, therefore, a complete characterization of their effect is needed if higher accuracy is required.

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BOOK REVIEW

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$89.75 (U.S. and Canada).
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This book is the updated proceedings of a symposium held in Toronto in October 1986 at the annual meeting of the American Association of Cereal Chemists. The fourteen chapters are the contribution of twenty three scientists. The work reported is substantially basic and serves to present views from several perspectives. The initial chapter by one of the editors, John Finley, provides an overview of the effects of processing on proteins and points to the work of the various contributions which follow. In Chapter 2, Finley and his associates look at water as a plasticizer of gluten and other protein polymers. An interesting section deals with the myth of "bound" water. The food engineer explaining dehydration will likely retain the simplistic explanation of "bound" water for some time to come. Until the model systems of the chemist realize comparable results, food scientists will reserve concensus. Meanwhile, water relations in food systems will continue to be a significant area of research. The 115 page chapter continues to discuss the importance of glass transition and the significance of nonequilibrium glassy and rubbery states to rheological properties. Again, the basic chemistry model and the whole food model need further coordinated research. Chapter 3 by Nakai and Li-Chan discusses the effects of heating on protein functionality with emphasis on protein hydrophobicity. Chapters 4 and 5 discuss toxicological aspects of protein foods. Arthur Miller discusses thermally induced mutagens in muscle protein foods. There is good agreement that mutagens are formed but the preventive role of antioxidants as well as the risk epidemiologically of such mutagens is in the very early stages of study. All America needs is the hysteria that would result if the carcinogens induced by various fast food company practices were advertised! Chapter 5 by Friedman, Gumbmann and Ziderman reviews the toxicology and implications of browning during *simulated* crust-baking. The formation of carcinogens do occur during the heating of plant material, however, there is a dearth of information. Sodium ascorbate in heated (200–215°C) systems with casein does not inhibit the growth of mice whereas the systems with heated soy protein and wheat gluten results in growth inhibition. Why ascorbic acid does not have this effect is not under-

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stood. Chapter 6 by Dixon Phillips is a comprehensive treatment of the effect of extrusion cooking on the *protein* nutritional quality of plant proteins. Chapter 7 by Michael Otterburn discusses natural protein crosslinking relevant to functional properties. Chapter 8 by Milton Feather deals with glucose-alanine interactions in Maillard polymer formation and Strecker degradations. The following Chapter (#9) by G-C Xen, T-C Lee and C. O. Chichester looks at the effect of Maillard browning reaction as examined by nine different assays ranging from color to SDS polyacrylamide gel. This systematic dissertation type study demonstrated the relationship of brown color, fluorescense development, available lysine, and dye binding in determining the degree of Maillard. Enzymes in addition to proteinases that affect protein quality is the topic of Chapter 10 by Ory, Flick and Cook. A technology to modify proteins with hydrophobic ligands thus changing protein functionality is described by Arai and Watanabe in Chapter 11. The texture of gelled muscle protein foods as affected by nonmuscle proteins as occurs in processed meat products is the topic of Foegeding and Lanier in Chapter 12. Wheat proteins on breadmaking quality is Chapter 13 by Bushuk and Macritchie and focuses on aspects of protein structure that differentiate wheat from other cereals and thus the unique capacity of wheat protein of form a dough. The functionality of the protein of soft wheat flour in cookie applications is by Kulp and Olewnick and serves as the closing chapter. This book is a must for the student of the role of chemistry in the functional transformations of processed protein foods. The nutritional aspects reported are restricted to protein quality *per se* and it is not comprehensive. The limited introduction of the toxicological issues that are emerging with the processing of protein foods may be of interest. The book is highly recommended to food chemists attempting to relate processing and changes in functional properties to underlying chemistry.

PAUL A. LACHANCE

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PUBLICATIONS IN FOOD SCIENCE AND NUTRITION

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Page two should contain an abstract of not more than 150 words. This abstract should be intelligible by itself.

The main text should begin on page three and will ordinarily have the following arrangement:

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Materials and Methods: Enough information should be provided to allow other investigators to repeat the work. Avoid repeating the details of procedures which have already been published elsewhere.

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Discussion: The discussion section should be used for the interpretation of results. The results should not be repeated.

In some cases it might be desirable to combine results and discussion sections.

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DEWALD, B., DULANEY, J.T. and TOUSTER, O. 1974. Solubilization and polyacrylamide gel electrophoresis of membrane enzymes with detergents. In *Methods in Enzymology,* Vol. xxxii, (S. Fleischer and L. Packer, eds.)pp. 82–91, Academic Press, New York.

HASSON, E.P. and LATIES, G.G. 1976. Separation and characterization of potato lipid acylhydrolases. Plant Physiol. *57,* 142–147.

ZABORSKY, O. 1973. *Immobilized Enzymes,* pp. 28–46, CRC Press, Cleveland, Ohio

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Table 1. Activity of potato acyl-hydrolases on neutral lipids, galactolipids, and phospholipids.

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