JOURNAL OF FOOD PROCESSING AND PRESERVATION

D.B. LUND
EDITOR

FOOD & NUTRITION PRESS, INC.

VOLUME 14, NUMBER 2

APRIL 1990

JOURNAL OF FOOD PROCESSING AND PRESERVATION

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All subscriptions and inquiries regarding subscriptions should be sent to Food & Nutrition Press, Inc., P.O. Box 374, Trumbull, Connecticut 06611 USA.

One volume of six issues will be published annually. The price for Volume 14 is $117.00 which includes postage to U.S., Canada, and Mexico. Subscriptions to other countries are $134.00 per year via surface mail, and $143.00 per year via airmail.

Subscriptions for individuals for their own personal use are $97.00 for Volume 14 which includes postage to U.S., Canada, and Mexico. Personal subscriptions to other countries are $114.00 per year via surface mail, and $123.00 per year via airmail. Subscriptions for individuals should be sent direct to the publisher and marked for personal use.

The *Journal of Food Processing and Preservation* is listed in *Current Contents/Agriculture, Biology & Environmental Sciences (CC/AB).*

The *Journal of Food Processing and Preservation* (ISSN: 0145-8892) is published bimonthly by Food & Nutrition Press, Inc. — Office of Publication is 6527 Main Street, Trumbull, Connecticut 06611 USA.

Second class postage paid at Bridgeport, CT 06602.

POSTMASTER: Send address changes to Food & Nutrition Press, Inc., P.O. Box 374, Trumbull, CT 06611.

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FOOD & NUTRITION PRESS, INC.
TRUMBULL, CONNECTICUT 06611 USA

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ISSN 0145-8892

Printed in the United States of America

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METHYL BROMIDE, TIME AND TEMPERATURE OF EXPOSURE ON APPLE QUALITY

S. R. DRAKE[1], H. R. MOFFITT[2] and J. P. MATTHEIS[1]

Accepted for Publication May 3, 1989

ABSTRACT

The use of methyl bromide (MeBr) as a fumigant to control codling moth in 'Delicious' apples resulted in a loss of firmness, internal color and therefore a reduction in the amount of acceptable fruit. Time and temperature of MeBr exposure were directly related to firmness and internal color loss. As the exposure time was increased beyond 2 h and exposure temperature above 6°C firmness and internal color loss were accelerated. An 8-day ambient storage period exacerbated firmness and internal color loss as time and temperature of MeBr exposure were increased. A fumigation regime of 56 g MeBr/m³ at 6°C for 2 h resulted in acceptable fruit during a 60 day refrigerated storage period. Increased exposure times or temperatures beyond 56 g MeBr/m³ at 6°C for 2 h resulted in unacceptable firmness and internal color loss, coupled with a major loss in acceptable fruit.

INTRODUCTION

Exportation of apples ('Delicious') to foreign markets is a major priority of the Washington State apple industry. One of the primary factors restricting export of apples are quarantine barriers established to prevent the spread of the codling moth (*Cydia pomonella* L.). Methyl bromide (MeBr) has been used as a fumigant for disinfecting fruit with varying degrees of success. A major disadvantage of using MeBr is that high dosages required to eliminate the insect in question often lead to injury of the host fruit.

MeBr injury to apples (*Malus domestica* Borkh.) has been widely reported, but results have varied due to many reasons, including the cultivar, fumigation procedures and the length of time required for the injury to appear (Phillips *et al.* 1938; Phillips and Monro 1939; Chapman 1940; Kenworthy 1944; Kenworthy and Gaddis 1946; Claypool and Vines 1956; Olsen 1986; Drake *et al.* 1988).

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Journal of Food Processing and Preservation **14** (1990) 85-92.

Phillips and Monro (1939) observed no injury to 'Delicious' apples when treated with MeBr. Kenworthy and Gaddis (1946) observed internal and external injury to 'Delicious' apples treated with MeBr. Olsen (1986) observed internal browning in 'Delicious' apples after treatment with high rates of MeBr. Drake *et al.* (1988) reported internal darkening of 'Delicious' apples after exposure to MeBr, but the damage required time to appear and was dosage and possibly temperature and time of exposure dependent.

After the initial study (Drake *et al.* 1988) a regime of 56 g MeBr/m^3 @ 6°C for 2 h was determined to be the optimum treatment procedure based on codling moth mortality and lack of fruit injury. The possibility exists that during commercial fumigation either the fumigation temperature or exposure time may be altered to meet contingencies. This study was initiated to determine the effect of 56 g/m^3 of MeBr at varying temperature and exposure times.

MATERIALS AND METHODS

Twenty-seven loose packed boxes of Fancy 'Delicious' apples, size 113, were harvested in September and stored in a commercial controlled atmosphere (CA) storage facility (1.5% O_2; 2% CO_2) until late February. Apples were fumigated with MeBr at either 0 or 56 g/m^3 at 6°, 10°, or 14°C for either 2, 2.5 or 3 h. Fumigations were conducted as previously reported (Drake *et al.* 1988). After fumigation the apples were boxed (no liner), placed in refrigerated storage at 0°C, periodically removed (<7, 30 and 60 days) and evaluated.

A sample of 18 apples of each combination of MeBr (2), temperature (3) and exposure time (3) was examined after each storage period. Nine apples were evaluated immediately after removal from storage, the remaining 9 apples were evaluated after 8 days at ambient temperature. Fruit were evaluated for firmness, objective and subjective internal color and titratable acidity. Evaluations were conducted as previously reported (Drake *et al.* 1988). Analysis of variance was determined by SAS (1985) and means were separated by the Waller-Duncan test.

RESULTS AND DISCUSSION

Firmness of MeBr-treated 'Delicious' apples was reduced compared to apples that were not fumigated (Table 1). Although firmness of both MeBr-treated and nontreated apples declined over time, firmness loss of MeBr-treated apples was 23% compared to only 10% for nontreated apples. Time and/or temperature of MeBr exposure along with storage duration resulted in reduced firmness. As time of MeBr exposure was increased from 2.0 to 3.0 h firmness declined approximately 6%. There was no statistical difference in firmness between 2.0 and 2.5 h or 2.5 and 3.0 h of exposure. A steady decline in firmness was evident as the

TABLE 1.
QUALITY ATTRIBUTES OF 'DELICIOUS' APPLES AS INFLUENCED BY THE MAIN EFFECTS OF MeBr, TIME, TEMPERATURE, COLD STORAGE AND AMBIENT STORAGE

Treatment MeBr	Firmness (N)	Agtron Internal flesh color Green	Blue	Visual Color	Titratable acidity (%) Malic	(%) Good Fruit
Control	60.1 A[1/]	36.2 A	25.4 A	1.3 B	0.19 B	98.5 A
56 g/m^3	55.6 B	30.0 B	22.1 B	1.9 A	0.21 A	79.8 B
Exposure time						
2.0 hrs	60.9 A	35.0 A	24.3 NS	1.4 C	0.20 NS	94.3 A
2.5	57.9 AB	31.3 B	23.7	1.5 B	0.20	88.4 B
3.0	57.1 B	33.0 AB	23.2	1.8 A	0.20	84.8 B
Exposure temperatures						
6°C	59.2 A	36.9 A	25.4 A	1.3 C	0.20 NS	97.5 A
10°	58.0 B	32.2 B	23.2 B	1.5 B	0.20	87.8 B
14°	56.4 C	30.3 C	22.7 B	1.9 A	0.20	82.2 C
Storage, cold						
30 days	57.8 NS	32.1 B	22.7 B	1.6 NS	0.20 NS	90.3 NS
60	58.0	34.1 A	24.8 A	1.6	0.20	88.0
Ripening						
0 days	63.0 A	36.0 A	27.2 A	1.3 B	0.21 A	98.1 A
8	52.7 B	30.3 B	20.3 B	1.9 A	0.19 B	80.2 B

[1/]Means in a column within treatments not followed by the same letter are significantly different ($P > 0.05$).

exposure temperature was increased by 4° increments. This decline in firmness amounted to 2% for every 4° rise in temperature. Drake *et al.* (1988) in an earlier study also reported firmness losses as temperature of exposure was increased, but in that particular study comparisons were between 6° and 20°. Storage of apples at ambient temperature, to simulate marketing conditions and ripen the fruit, also resulted in a reduced firmness of the apples of approximately 16%.

Firmness loss of 'Delicious' apples was related to the interaction of MeBr, exposure time and storage at ambient temperature for 8 days (Table 2). No firmness loss was noted for either MeBr-treated or nontreated apples at either 2.0, 2.5 or 3.0 h of exposure when immediately removed from cold storage. After 8 days at ambient temperature there were significant differences between MeBr-treated and nontreated apples. As the length of exposure time increased from 2.0 to 2.5 h there was a distinct increase in firmness loss between MeBr-treated and nontreated fruit from 10 to 18%. This loss in firmness between MeBr-treated and nontreated apples remained the same as the length of exposure was increased from 2.5 to 3.0 h.

Firmness loss was also related to the interaction between MeBr, temperature of exposure and storage at ambient temperature for 8 days (Table 2). Again, no firmness losses were evident when apples were immediately removed from cold storage, but after 8 days at ambient storage firmness declined (17 to 24%) for fruit treated with MeBr with exposure temperatures of 10° and 14°, respectively. Although not significant, there was a 4% decrease in firmness for MeBr-treated fruit with an exposure temperature of 6°.

Internal flesh color (objective and subjective) was reduced or darkened in 'Delicious' apples after exposure to MeBr, and these changes were proportional to exposure time and exposure temperature but only when apples were held at ambient temperature for 8 days (Table 1). Internal color change in 'Delicious' apples both by objective and subjective measurements was directly related to the interaction of MeBr, time and temperature of exposure and cold storage (Table 3). No change in the internal color (objective or subjective) over the entire 60 days of cold storage was evident when apples were fumigated with MeBr at 6° for either 2.0, 2.5 or 3.0 h. When the temperature of MeBr exposures was increased to 10°, a darkening of the internal color of 'Delicious' apples was present only after 30 days of storage regardless of the length of exposure. This darkening of internal color due to MeBr exposure was even more pronounced after the 14° exposure temperature and 30 days of cold storage. At either 10° or 14° exposure, as the length of exposure time was increased the internal color difference between the control and fumigated fruit increased. When the 2 extremes (10°/2.0 h or 14°/3.0 h) are compared after 30 days of storage, a 22% to 54% reduction in Agtron green color and a 30 to 45% reduction in Agtron yellow was noted.

TABLE 2.
FIRMNESS OF 'DELICIOUS' APPLES BEFORE AND AFTER AMBIENT STORAGE AS INFLUENCED BY THE INTERACTION OF MeBr WITH TIME AND MeBr WITH TEMPERATURE

MeBr	Exposure time (hrs)	Days at Ambient Storage 0	8	Exposure Temp (°C)	Days at Ambient Storage 0	8
Control	2.0	63.9 A[1/]	56.5 A	6°	63.7 A	56.5 A
56 g/m³		63.0 A	50.9 B		62.4 A	54.2 A
Control	2.5	63.2 A	58.1 A	10°	63.3 A	57.5 A
56 g/m³		62.5 A	47.9 B		63.6 A	47.5 B
Control	3.0	62.6 A	56.4 A	14°	62.6 A	57.1 A
56 g/m³		62.9 A	46.5 B		62.4 A	43.6 B

[1/]Means in a column within treatments not followed by the same letter are significantly different (P > 0.5).

TABLE 3.
OBJECTIVE AND SUBJECTIVE COLOR AND PERCENTAGE OF GOOD FRUIT INFLUENCED BY INTERACTION OF EXPOSURE TIME AND TEMPERATURE OF MeBr TREATMENT, LENGTH OF COLD AND AMBIENT TEMPERATURE STORAGE

Temperature and time of exposure (hrs/C°)	MeBr treatment	Agtron Internal flesh color Green 30 days	Agtron Internal flesh color Green 60 days	Agtron Internal flesh color Blue 30 days	Agtron Internal flesh color Blue 60 days	Visual[z] Color 30 days	Visual[z] Color 60 days	% Acceptable Fruit 0 days	% Acceptable Fruit 8 days
6°/2.0 hrs	Control	37.2 A[y/]	33.2 A	23.2 A	22.8 A	1.3 A	1.1 A	97.9 A	100.0 A
	56 g/m³	35.9 A	37.9 A	24.1 A	27.9 A	1.4 A	1.2 A	97.9 A	97.2 A
6°/2.5 hrs	Control	37.6 A	37.1 A	23.1 A	28.2 A	1.3 A	1.1 A	100.0 A	100.0 A
	56 g/m³	37.0A	39.0 A	25.8 A	27.0 A	1.6 A	1.4 A	93.1 A	95.6 A
6°/3.0 hrs	Control	36.3 A	38.5 A	24.6 A	28.4 A	1.4 A	1.2 A	100.0 A	100.0 A
	56 g/m³	37.1 A	35.5 A	22.9 A	25.7 A	1.6 A	1.2 A	94.5 A	93.7 B
10°/2.0 hrs	Control	36.1 A	39.1 A	27.3 A	29.2 A	1.1 A	1.3 A	97.6 A	100.0 A
	56 g/m³	28.3 B	37.6 A	19.0 B	22.2 B	1.6 B	1.4 B	97.2 A	83.8 B
10°/2.5 hrs	Control	33.0 A	36.2 A	21.8 A	24.9 A	1.2 A	1.3 A	97.6 A	93.5 A
	56 g/m³	28.3 B	26.3 B	20.2 A	21.6 A	1.3 A	2.1 B	100.0 A	52.7 B
10°/3.0 hrs	Control	34.1 A	39.0 A	23.9 A	29.6 A	1.2 A	1.1 A	97.6 A	96.6 A
	56 g/m³	23.5 B	24.2 B	19.3 A	19.1 B	2.3 B	2.2 B	97.2 A	38.7 B
14°/2.0 hrs	Control	33.2 A	38.2 A	24.0 A	26.8 A	1.5 A	1.3 A	100.0 A	97.6 A
	56 g/m³	33.5 A	29.2 B	25.0 A	20.4 B	1.7 A	2.1 B	100.0 A	61.8 B
14°/2.5 hrs	Control	31.0 A	38.0 A	25.0 A	26.3 A	1.4 A	1.4 A	100.0 A	97.6 A
	56 g/m³	27.2 A	26.0 B	19.9 B	19.9 B	2.1 B	2.2 B	94.5 A	38.7 B
14°/3.0 hrs	Control	33.8 A	40.3 A	25.2 A	30.1 A	1.6 A	1.3 A	100.0 A	97.6 A
	56 g/m³	15.4 B	17.9 B	13.9 B	15.8 B	2.8 B	3.1 B	100.0 A	< 1.0 B

[y/] Visual color rate on a scale of (1=no discoloration; 5=severe discoloration).

[z/]Means in a column within treatments not followed by the same letter are significantly different ($P > 0.05$).

Neither MeBr exposure duration nor cold storage influenced the acidity of 'Delicious' apples (Table 1). The use of MeBr did result in a slight increase in acidity (10%) when compared to nonfumigated apples. A 10% reduction in acidity was noted after an 8 day ambient temperature storage period. In a previous study (Drake *et al.* 1988) noted no difference in the acidity of 'Delicious' apples when exposed to MeBr. Apparently, MeBr fumigation has little if any effect on the acidity of 'Delicious' apples.

Regardless of other quality attributes the percent of marketable fruit in a lot is the determining factor in acceptability. In this study MeBr fumigation with increased length and time of exposure, and storage at ambient temperature reduced the amount of marketable or acceptable apples (Table 1). As exposure time was increased from 2.0 to 2.5 h the percentage of marketable apples was reduced. There was no significant reduction in the number of marketable apples when exposure time was increased from 2.5 to 3.0 h. As the temperature of MeBr fumigation was increased (6° to 14°) there was a corresponding reduction ($r^2 = 0.9$) in the percentage of marketable apples. An 8 day storage period at ambient temperature also resulted in an 18% reduction in the amount of marketable apples.

There was direct interaction between MeBr, time and length of exposure and storage at ambient temperature for 8 days (Table 3). Before an 8 day ambient temperature storage 95% of all apples regardless of MeBr fumigation or time and temperature of exposure were good or acceptable. After an 8 day ambient storage period only those apples treated with MeBr at 6° for 2.0 or 2.5 h were similar to the control apples in acceptability. The amount of good apples fumigated at either 10° or 14° regardless of exposure time (2.0, 2.5 or 3.0 h) was greatly reduced when compared to control fruit. The percent of good fruit was reduced from 93% to less than 1% as the temperature and time of MeBr exposure was increased from 6° for 3.0 h to 14° for 3.0 h, respectively.

CONCLUSIONS

The use of MeBr as a fumigant for apples does result in a loss of firmness. This loss in firmness is accentuated after ambient storage and as the time and temperature of exposure to MeBr is increased. Ambient storage coupled with a time exposure duration greater than 2.0 h and a temperature of 10° or 14° reduced the firmness of the fumigated apples below the Washington State export standard.

Exposure of 'Delicious' apples to MeBr at temperatures of 10° or more resulted in a darkening of the internal color. This darkening of the apples after MeBr exposure was not only evident by objective measurement but subjective measurement as well. The darker internal color displayed by apples after MeBr exposure would eliminate, in most instances, those apples from the market chain.

This was particularly obvious in this study when number of marketable fruit was considered.

Fumigation of 'Delicious' apples with MeBr at 6° for 2.0 h resulted in an acceptable product. But, if the time or temperature of MeBr exposure was increased acceptability of the fumigated apples decreased to an unacceptable level when either firmness or internal color of the fruit is considered.

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A RESPONSE SURFACE METHODOLOGY APPROACH TO OPTIMIZE POTATO DEHYDRATION PROCESS

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Accepted for Publication May 3, 1989

ABSTRACT

A process for the production of high quality dehydrated potato cubes by high temperature fluidized bed (HTFB) initial drying followed by tunnel drying was optimized by response surface methodology. Drying temperature (T), exposure time in HTFB drier (t) and concentration of biopolymers (C) as pretreatment were determined as the most important factors affecting rehydration ratio, puffing, nonenzymatic browning and water holding capacity in the finished product. Optimum conditions for 0.95 cm potato cubes were: T = 145°C; t = 10 min; C = 1.2% and blanching time (b) = 4.5 min. Values predicted by the surface response model for rehydration ratio, bulk density, nonenzymatic browning and expressible fluid at the optimum were 5.75, 0.156, 0.20 and 4.8%, respectively. These values were experimentally varified and very close agreement between experimental and predicted values were obtained.

INTRODUCTION

Increased consumption of dried vegetables in products such as instant soups is increasing demand for dehydrated potatoes in the food industry. Defense agencies procure substantial amounts of dehydrated diced potatoes for military ration. The dehydrated potato cubes which are commercially available are not of prime quality, and a need exists to optimize the quality of this product.

Color and rehydration ratio are very important quality attributes of the dehydrated product. The browning reaction is often accelerated during drying and can lead to reduction in visual and organoleptic quality, and nutritional value of the dried product (Talburt and Smith 1987). The rate of the browning reaction is strongly affected by the temperature and moisture existing in the potato dice during drying (Hendel *et al.* 1955). Labuza (1973) suggested that suitable changes in drying conditions would alter the moisture-temperature profile and may produce a better product. Islam and Flink (1982) studied the effect of air velocity

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Journal of Food Processing and Preservation **14** (1990) 93–106.

on the dehydration efficiency of potato dices. They showed that the dehydration rate increases with the increase in air velocity. Islam and Flink (1982) removed part of the moisture from potato slices by osmotic dehydration in 45% sucrose/15% salt solution and then dried in a conventional drier at 65°C with an air flow of 2.5 m/s. Katara and Nath (1985) blanched potato cubes in 2% sodium chloride as well as in 1% potassium meta-bisulfite (KMS) solution for 4.5 to 6.5 min and dried the cubes in a cabinet drier. They recommended a drying temperature of 84°C for the first 4 h and then at 74°C until a final moisture content of 5–6% is reached. The dried potato samples had rehydration ratio (RR) of 4.8 or less. The KMS pretreated samples were better in color than sodium chloride treated samples. Aguilera *et al.* (1974) showed that moisture removal and browning development are related, as both proceed more rapidly with an increase in temperature although the rates were different. Mishkin *et al.* (1983) optimized a dehydration process for potatoes using a simple air drier, with the objective of minimizing browning. They recommended a drying temperature of 73°C until the final moisture was 5–6%.

The purpose of this study was to establish the most appropriate drying conditions, concentrations of biopolymers used as pretreatment and the blanching time for the production of high quality dehydrated potato cubes having maximum rehydration ratio, puffing, water holding capacity and minimum nonenzymatic browning.

MATERIALS AND METHODS

Potato Processing

The potatoes of Russel Burbank variety were obtained from local market. The potatoes were washed, peeled manually and diced into 0.95 cm cubes using a model TR-22 dicer (Dito Dean). The diced potatoes containing initially 78% moisture, were blanched in water containing a mixture of biopolymers, which were pectin and polydextrose in a 1:5 ratio, at concentrations shown in Table 1. Dehydration was preformed using two different types of driers in sequential steps. The first drying step was utilizing a batch type high temperature fluidized bed drier (HTFB) at temperature and exposure time given in Table 1. Ambient air used for drying was at 20°C and 20% relative humidity, therefore the wet bulb temperature of the drying air was 36 and 41°C at 135 and 155°C, respectively. The partially dried potato cubes were immediately transfered to a tunnel drier at 70°C and 4 m/s air velocity and dried to a final moisture level of 5–6%.

Experimental Design

Based on literature (Aguilera *et al.* 1975; Mudahar *et al.* 1989), the drying temperature, dehydration time, blanching time and pretreatment concentration

TABLE 1.
CODING OF LEVELS OF INDEPENDENT VARIABLES USED IN DEVELOPING EXPERIMENTAL DATA FOR OPTIMIZATION OF THE PROCESS FOR DEHYDRATION OF POTATO CUBES IN A HIGH TEMPERATURE FLUIDIZED BED (HTFB) DRIER

Independent variables	Symbols		Levels	
	Uncoded	Coded	Uncoded	Coded
Drying Temp. in HTFB, (°C)	T	X1	155	1
			145	0
			135	-1
Exposure time in HTFB, (min.)	t	X2	15	1
			10	0
			5	-1
Concentration of biopolymers, (%)[1]	C	X3	1.5	1
			1.0	0
			0.5	-1
Blanching time, (min.)	b	X4	6	1
			4	0
			2	-1

[1]Biopolymers used were pectin and polydextrose in a 1:5 ratio.

were considered as the most important factors affecting the quality of dehydrated potato cubes. Quality attributes important in the finished product are: rehydration ratio, puffing, nonenzymatic browning and water holding capacity. Response surface methodology (RSM) was used to optimize this complex process. RSM has been used by several investigators in optimizing food process operations (Smith *et al.* 1977; Lah *et al.* 1980; Floros and Chinnan 1987, 1988; Mudahar *et al.* 1989). A three level four factor design was adopted (Box and Behnken 1960) to optimize the process for the dehydration of potato cubes. It was assumed that four mathematical functions, f_k (k = 1, 2, 3, 4), existed for each response variable Y_k rehydration ratio (RR), bulk density (BD), nonenzymic browning (NB) and expressible fluid (EF) in the terms of four independent processing factors. Nonexpressible fluid was used as an indicator of water holding capacity.

$$Y_k = f_k (T, t, C, b) \quad (1)$$

Where:
T = Drying temperature in HTFB
t = Exposure time in HTFB
C = Concentration of biopolymers
b = blanching time

To approximate the function f_k, second degree polynomial equations were used:

$$y_k = B_{ko} + \sum_{i=1}^{4} B_{ki}x_i + \sum_{i=1}^{4} B_{kii}\, x_i^2 + \sum_{i=1}^{3}\sum_{j=i+1}^{4} B_{kij}x_i x_j$$

Where B_{ko}, B_{ki}, B_{kii} and B_{kij} are constant coefficients and X_i's are the coded independent variables, linearly related to T, t, C and b. Statistical Analysis System (SAS 1985) was used to fit the second order polynomial equation to the experimental data shown in Table 2.

TABLE 2.
EXPERIMENTAL DATA FOR THE REHYDRATION RATIO (RR), BULK DENSITY (BD), NONENZYMATIC BROWNING (NB) AND EXPRESSIBLE FLUID (EF) UNDER DIFFERENT TREATMENT CONDITIONS (CODED, REFER TO TABLE 1 FOR CODING) OF DRYING TEMPERATURE IN THE FLUIDIZED BED, DRYING TIME, BLANCH TIME AND CONCENTRATION OF BIOPOLYMER IN THE BLANCH SOLUTION

Treatment* #	Drying temp.	Expos. time	Conc.	Blanch. time	RR	BD	NB (OD)	EF (%)
1	1	1	0	0	5.40	0.150	0.45	6.39
2	1	-1	0	0	4.86	0.188	0.15	5.24
3	-1	1	0	0	4.72	0.165	0.12	6.88
4	-1	-1	0	0	4.81	0.215	0.06	5.26
5	0	0	1	1	5.88	0.158	0.29	3.43
6	0	0	1	-1	6.06	0.165	0.30	3.91
7	0	0	-1	1	5.80	0.155	0.28	7.16
8	0	0	-1	-1	5.65	0.174	0.24	5.56
9	0	0	0	0	5.84	0.163	0.22	5.62
10	1	0	0	1	5.61	0.162	0.27	6.33
11	1	0	0	-1	5.11	0.173	0.22	4.84
12	-1	0	0	1	4.43	0.177	0.08	4.74
13	-1	0	0	-1	4.24	0.180	0.10	3.50
14	0	1	1	0	5.35	0.157	0.30	3.80
15	0	1	-1	0	4.96	0.174	0.42	6.30
16	0	-1	1	0	4.54	0.192	0.09	6.14
17	0	-1	-1	0	4.52	0.206	0.09	3.77
18	0	0	0	0	5.71	0.156	0.16	5.70
19	1	0	1	0	5.34	0.162	0.35	3.35
20	1	0	-1	0	5.42	0.153	0.38	4.04
21	-1	0	1	0	4.69	0.163	0.09	2.35
22	-1	0	-1	0	4.47	0.164	0.08	5.22
23	0	1	0	1	4.61	0.142	0.27	4.14
24	0	1	0	-1	4.51	0.145	0.25	3.92
25	0	-1	0	1	4.80	0.201	0.07	4.12
26	0	-1	0	-1	4.83	0.202	0.07	4.76
27	0	0	0	0	5.60	0.152	0.19	4.79

* Experimental runs were performed in random order.

Analysis

Dehydrated potato cubes were analyzed for rehydration ratio, puffing, nonenzymatic browning and water holding capacity. Until the analysis was performed, these were stored in opaque, air tight plastic bottles. Rehydration ratio was measured as the total mass of rehydrated potato per unit weight of dry matter after rehydration. Rehydration was done by boiling 2 g of dried potato sample in 100 mL distilled water for 30 min. Nonenzymatic browning was measured by a method of Baloch *et al.* (1981). Dehydrated potato was extracted with 2% acetic acid, mixed with equal volume of ethanol, and centrifuged. The absorbance of supernatant was measured at 420 nm on a Spectronic-70. Bulk density, used as an index of puffing, was determined on weight per volume basis (Kim and Toledo 1987). A 100 mL graduated cylinder was used and the weight of dehydrated potatoes needed to fill the cylinder to the 100 mL mark was determined. Free or mobilizable water was evaluated as a textural parameter, an index of water holding capacity. More free fluid expressed on compression of a potato cube indicated poor water holding capacity and vice versa. The procedure reported by Grau and Hamm (1957) was modified and used for the measurement of EF. Individual potato cubes weighing approximately 1 g each were positioned on a piece of filter paper and uniaxially compressed to 50% deformation in an Instron universal testing machine. The expressible fluid was absorbed by filter paper. The reduction in weight of potato cube was expressed as percentage expressible fluid.

Verification

Optimum processing conditions in terms of drying temperature, exposure time in HTFB, concentration of biopolymers and blanching time were calculated using the prediction equations derived by RSM. Potato cubes were processed using the optimum conditions. The product thus obtained was analysed for RR, BD, NB and EF and experimental values were compared to those predicted by the model.

RESULTS AND DISCUSSION

The regression coefficients (B_{ki}) of the second degree polynomial are given in Table 3. The analysis of variance for the four response variables (Table 4) indicated that the model developed for the bulk density, nonenzymatic browning and expressible fluid appear to be very adequate, possessing no lack of fit and satisfactory values of R^2. However, rehydration ratio possessed lack of fit at 10% level of significance.

From the statistical analysis (Table 5), the exposure time (t) was shown to be the most important factor because it affected RR, NB and BD significantly.

TABLE 3.
VALUE OF THE REGRESSION COEFFICIENTS OF THE SECOND ORDER POLYNOMIALS REPRESENTING THE RELATIONSHIP BETWEEN THE INDICATED RESPONSE VARIABLES (Y_k) AND THE INDEPENDENT VARIABLES OF DRYING TIME IN THE FLUIDIZED BED (i OR j = 1), EXPOSURE TIME (i OR j = 2), CONC. OF BIOPOLYMERS IN THE BLANCH SOLUTION (i OR j = 3), AND BLANCH TIME (i OR j = 4)

Coefficients	Rehydration Ratio (R) k = 1	Bulk Density (BD) k = 2	Nonenzymatic Browning (NB) k = 3	Expressible Fluid (EF) k = 4
b_{k0}	5.716	0.157	0.190	5.370
b_{k1}	0.365	-0.006	0.108	0.187
b_{k2}	0.099	-0.023	0.107	0.178
b_{k3}	0.087	-0.003	-0.005	-1.151
b_{k4}	0.061	-0.004	0.006	0.286
b_{k11}	-0.496	0.006	-0.010	-0.215
b_{k21}	0.158	0.003	0.060	-0.118
b_{k22}	-0.645	0.017	-0.011	0.107
b_{k31}	-0.075	0.003	-0.010	0.545
b_{k32}	0.093	-0.001	-0.030	0.033
b_{k33}	-0.033	0.002	0.060	-0.604
b_{k41}	0.083	-0.002	0.018	0.063
b_{k42}	0.057	0.001	0.005	0.215
b_{k43}	-0.083	0.003	-0.013	-0.520
b_{k44}	-0.193	0.004	-0.001	-0.432

$$* \quad Y_k = B_{ko} + \sum_{i=1}^{4} B_{ki}x_i + \sum_{i=1}^{4} B_{kii}\, x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} B_{kij}x_i x_j$$

Drying temperature (T) had a significant effect on nonenzymatic browning and rehydration ratio, but it did not effect the expressible fluid and bulk density significantly. Concentration of biopolymers was a significant factor for expressible fluid only, which indicates that the biopolymers improve the water holding capacity and thus the texture of rehydrated potato cubes. Blanching time was the least important factor as it did not effect any response variable significantly. Computer generated three dimensional plots for the most important independent variables (T and t), using the predictive equations for RR, BD, NB and EF (Fig. 1) provide an accurate geometrical representation of the behavior of the response variables within the range of the independent variables included in the experimental design.

The optimum level of these variables was established using a procedure described by Mayers (1971) and Draper (1963). Stationary points (Table 6) were located in four dimensional space. The first series of plots (9 for each response) was generated by holding concentration of biopolymers and blanching time

TABLE 4.
ANALYSIS OF VARIANCE TABLE SHOWING THE EFFECT OF TREATMENT VARIABLES AS A LINEAR TERM, QUADRATIC TERM AND INTERACTIONS (CROSS PRODUCT) ON THE RESPONSE VARIABLES, REHYDRATION RATIO, BULK DENSITY, NONENZYMIC BROWNING AND EXPRESSIBLE FLUID

Source	Degree of Freedom	Sum of Squares			
		Rehydration Ratio (RR)	Bulk Density (BD)	Nonenzymatic Browning (NB)	Expressible Fluid (EF)
Model	14	5.128	0.009***	0.325***	23.331
Linear	4	1.851	0.007***	0.276***	17.673**
Quadratic	4	3.065**	0.001**	0.028*	3.128
Cross product	6	0.212	0.001	0.021	2.530
Residual	12	2.397	0.001	0.028	14.801
Lack of fit	10	2.368*	0.001	0.026	14.293
Pure error	2	0.029	0.000	0.002	0.508
% Variability Explained (R^2)		68.15	87.36	92.10	61.19

* Significant at 10% level ; ** 5% level; *** 1% level

TABLE 5.
ANALYSIS OF VARIANCE TABLE SHOWING THE SIGNIFICANCE OF THE EFFECT OF THE PROCESSING VARIABLES ON EACH OF THE INDICATED RESPONSE VARIABLES

Process variables	DF	Sum of Squares			
		Rehydration Ratio (RR)	Bulk Density (BD)	Nonenzymatic Browning (NB)	Expressible Fluid (EF)
Drying temp. (T)	5	3.051*	0.001	0.155***	1.925
Exposure time(t)	5	2.474*	0.008***	0.155***	0.68
Conc. of biopolymers (C)	5	0.187	0.001	0.024	20.114**
Blanching time(b)	5	0.298	0.001	0.003	3.256

* Significant at 10% level; ** 5% level; *** 1% level

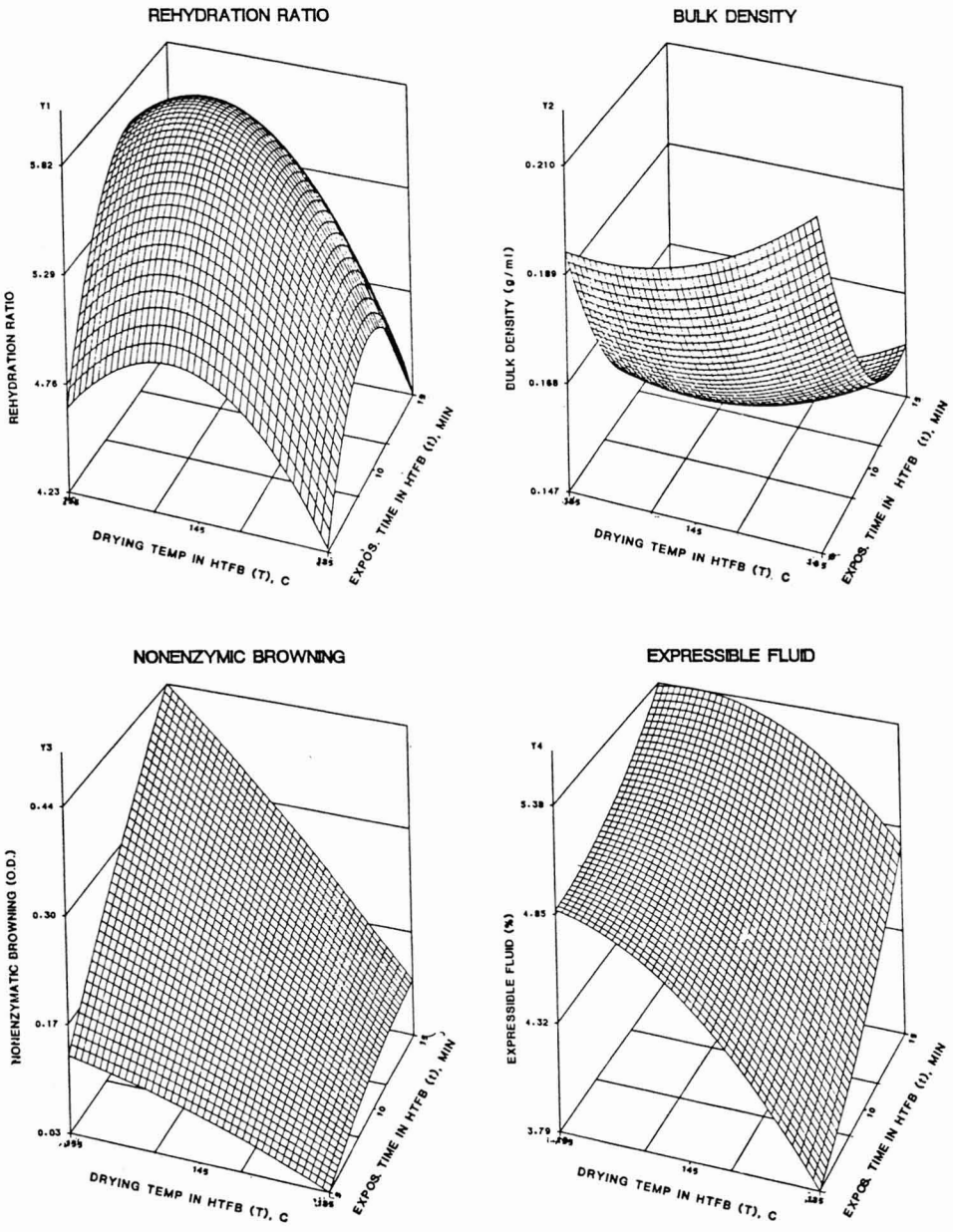

FIG. 1. A,B,C,D. RESPONSE SURFACES OF REHYDRATION RATIO, BULK DENSITY, NONENZYMATIC BROWNING AND EXPRESSIBLE FLUID AT CONSTANT BIOPOLYMER CONCENTRATION (1.2%) AND BLANCHING TIME (4.5 MIN)

TABLE 6.
PREDICTED LEVELS OF PROCESS VARIABLES YIELDING OPTIMUM RESPONSE OF REHYDRATION RATIO (RR), BULK DENSITY (BD), NONENZYMATIC BROWNING (NB) AND EXPRESSIBLE FLUID (EF)

Process variables	Levels for optimum response				Graphical optimum
	RR	BD	NB	EF	
Drying temp. (T)	0.35	0.46	-0.29	-0.84	0.00
Exposure time (t)	0.17	0.64	2.02	-2.02	0.00
Conc. of biopolymers (C)	0.71	-0.03	-0.93	-1.56	0.40
Blanching time(b)	0.09	0.63	-13.95	0.66	0.25

constant. The superimposed plots for all response variables are shown in Fig. 2. From these plots, the optimum dehydration temperature and exposure time in HTFB were 145°C and 10 min, respectively. The plots, corresponding to drying temperature of 145°C and exposure time of 10 min, gave maximum rehydration ratio and minimum bulk density. Least nonenzymatic browning and expressible fluid are presented in Fig. 3. Figure 3A indicates maximum rehydration ratio of about 5.72. Minimum values for bulk density, nonenzymatic browning and expressible fluid shown are about 0.156, 0.19 and 4.2, respectively (Fig. 3B, 3C and 3D).

The superimposed contour plot of Fig. 3A, 3B, 3C and 3D is shown in Fig. 4. A region, which fulfills the requirements of maximum rehydration ratio and minimum bulk density, becomes apparent. Values shown for nonenzymatic browning and expressible fluid are saddle point rather than a true minimum, but these values are close to the minimum values. This region (shaded in Fig. 4) of optimum response has RR, BD, NB and EF in the proximity of 5.75, 0.156, 0.20 and 4.80%, respectively. The combination of T, t, C and b values associated with the region of optimum response are also defined in Fig. 4.

Values of the independent variables, drying temperature of 145°C, exposure time in HTFB of 10 min, concentration of biopolymers of 1.2% and blanching time of 4.5 min were determined to be optimum by the RSM optimization procedure. The predicted optimum response values obtained were verified by processing potato cubes using this set of conditions. The predicted and experimental values for RR, BD, NB and EF at these conditions are shown in Table 7. The experimental values were very close to the predicted values for RR, BD

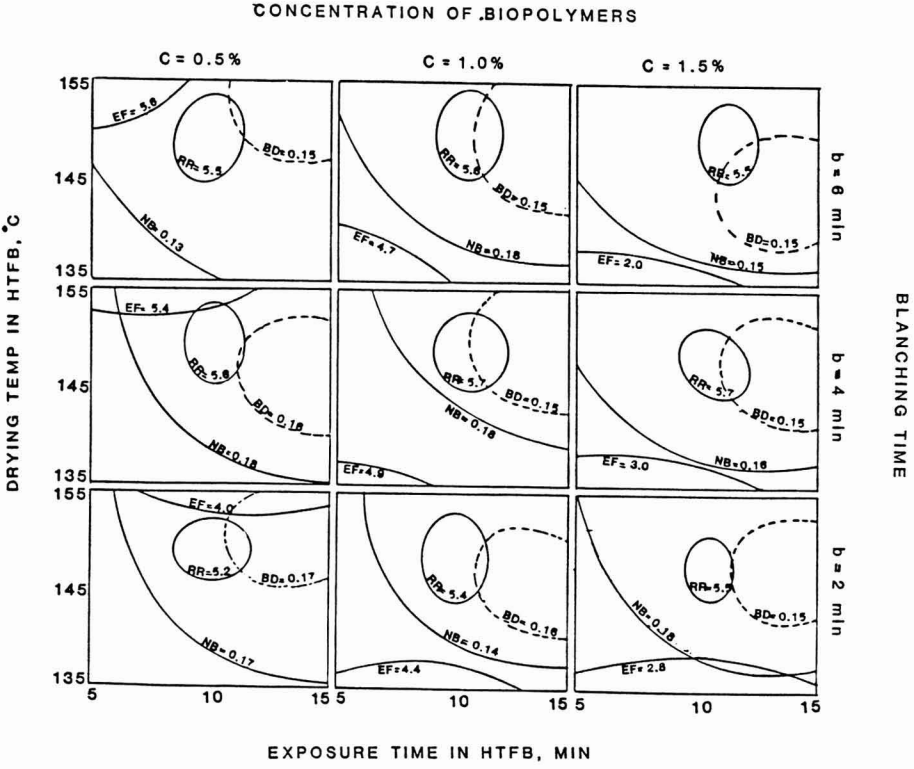

FIG. 2. SUPERIMPOSED PLOTS OF REHYDRATION RATIO, BULK DENSITY, NONENZYMIC BROWNING AND EXPRESSIBLE FLUID (%) AT DIFFERENT LEVELS OF INDEPENDENT VARIABLES

and EF but the experimental values for NB were much lower than the predicted values. The model equations proved to be good predictor of renydration ratio, bulk density and expressible fluid.

Simultaneous puffing and drying occurred in high temperature fluidized bed drying as evidenced by the low bulk density of the product. The biopolymers probably minimized tissue collapse during dehydration, which when combined with the porous structure, resulted in high moisture uptake on rehydration and reabsorption of the water of rehydation within the intact cell to improve the water holding capacity of the rehydrated product. Puffing requires rapid dehydration rates. Adequate time must be allowed in order that adequate drying develops to form a rigid structure which does not collapse on removal from the high temperature and subsequent low temperature drying to a shelf stable moisture content. Excessive exposure to high temperature results in nonenzymatic browning when the moisture content has dropped considerably to slow down evapo-

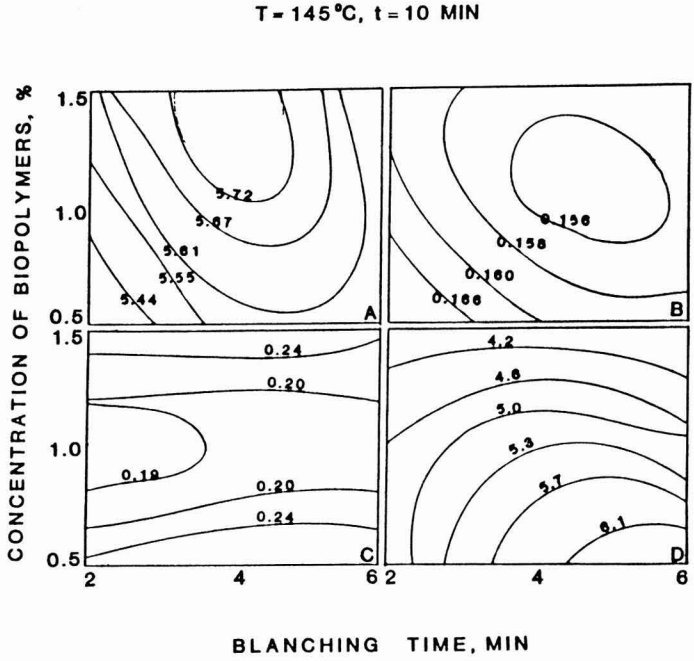

FIG. 3. CONTOUR PLOTS AT T = 145°C, t = 10 MIN FOR REHYDRATION RATIO, BULK DENSITY, NONENZYMATIC BROWNING AND EXPRESSIBLE FLUID

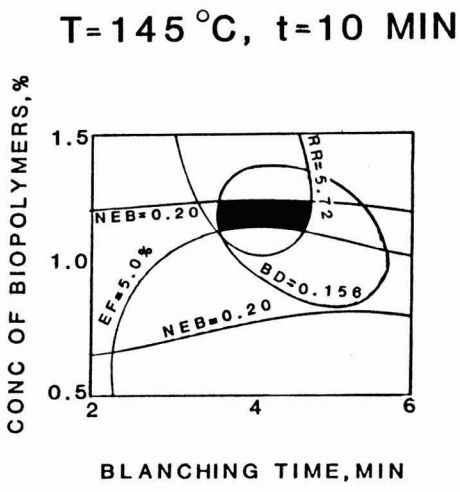

FIG. 4. SUPERIMPOSED PLOTS OBTAINED FROM FIG. 3

TABLE 7.
PREDICTED AND EXPERIMENTAL VALUES OF
THE RESPONSES AT OPTIMUM CONDITIONS

Response Variable	Predicted value	Experimental value*	
		Mean	Range
Rehydration Ratio (RR)	5.75	5.60	5.43 - 5.71
Bulk Density (BD)	0.156	0.127	0.125 - 0.130
Nonenzymatic Browning (NB)	0.20	0.122	0.110 - 0.130
Expressible Fluid (EF) (%)	4.80	3.91	2.62 - 6.11

* Results are of five replications. Optimum conditions are: drying temperature in high temperature fluidized bed (HTFB) of 145°C, exposure time of 10 min, biopolymer concentration in the blanch solution of 1.2% and blanch time of 4.5 min.

rative cooling and particle temperature increases above the wet bulb temperature of the drying air.

CONCLUSIONS

Response surface methodology was used to establish the optimum levels of independent factors, responsible for obtaining best quality dehydrated potato cubes. Predicted models for rehydration ratio, bulk density, nonenzymatic browning and expressible fluid were developed as function of drying temperature, exposure time in HTFB, concentration of biopolymers and blanching time. Processing conditions yielding an optimum process (maximum rehydration ratio and minimum bulk density, nonenzymatic browning and expressible fluid) for 0.95 cm cubes were determined as drying temperature in HTFB: 145°C; exposure time in HTFB: 10 min; concentration of biopolymers: 1.2%; and blanching time: 4.5 min.

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STORAGE BEHAVIOR OF AN EARLY GARLIC (*ALLIUM SATIVUM* L.) SUBJECT TO GAMMA-RAY RADIOINHIBITION

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Accepted for Publication May 3, 1989

ABSTRACT

Garlic bulbs of an early cultivar (Rosado Paraguayo) were irradiated with a dose of 50 Gy of Co-60 gamma rays at 30 days after harvest. Experimental lots were stored up to 300 days in a commercial warehouse (6–32°C; R.H. 40–50%). The treatment reduced the weight loss of the bulbs and increased the percentage of both marketable bulbs and cloves without affecting organoleptic properties. The results show that both the raw/fresh market for this early cultivar and the garlic processing industry would profit from the radioinhibition process.

INTRODUCTION

On the basis of sprouting inhibition ionizing radiation has proven to be useful to extend the shelf-life of garlic bulbs (El-Oksh *et al.* 1971; Croci and Curzio 1983; Thomas 1984). This application is referred to as a "radioinhibition" process, which has been approved by many countries, including Argentina (IAEA 1988). Also, data are presently available concerning with the associated physiological and biochemical aspects (Michiels 1967; El-Oksh *et al.* 1971; Croci *et al.* 1987; Croci 1988).

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Journal of Food Processing and Preservation **14** (1990) 107–112.

An early garlic cultivar (Rosado Paraguayo) has particular economic importance in the province of Córdoba (Argentina) due to its industrial processing utilization as well as its raw consumption. The postharvest physiological performance of this cultivar has been studied (Argüello *et al.* 1983; Argüello 1987) but no work has been done regarding its postharvest preservation by ionizing radiation.

The present study was undertaken to investigate the influence of the radioinhibition process on the postharvest behavior of garlic bulbs of *cv.* Rosado Paraguayo during commercial storage. A study of weight loss, bulb and clove marketability, visual dormancy rate (Burba *et al.* 1983) and sensory quality were made.

MATERIALS AND METHODS

The Rosado Paraguayo garlic cultivar grown in the N. W. of the province of Córdoba was used in this study. Garlic harvested in the middle of November was naturally cured in the field for 10 days before treatment. About 150 kg of sound and uniformly-sized bulbs were packed in carton boxes of $28 \times 30 \times 35$ cm size, equal to the capacity of the irradiation chamber. The treatment was applied 30 days after harvest as reported by Croci and Curzio (1983). Irradiation was carried out at the facilities of Comisión Nacional de Energía Atómica in Ezeiza Atomic Center, in the Transportable Irradiator IMO-I. The bulbs were treated with a dose of 50.0 Gy of 60-Co gamma rays at the rate of 0.35 Gy/s and a dose uniformity ratio of 1.25.

After treatment the bulbs were distributed in 72 netted nylon bags and their initial weight was recorded. The irradiated and control samples were stored in a commercial warehouse for 300 days at temperature that ranged from 6°C to 32°C and R.H. of 40–50%. The following data were recorded monthly on 8 bags taken at random: (1) The cumulative percentage mean weight loss starting 60 days after harvest. (2) The percentage of marketable bulbs starting 90 days after harvest. Bulbs that showed external sprouting, rotting or softening were discarded and the remainder were designated as marketable bulbs. (3) On the cloves from the second and third layers of marketable bulbs the percentage of marketable cloves was determinated with the same criterion used for marketable bulbs. (4) The growth of the inner sprout leaf was measured on 30 marketable cloves using the visual dormancy rate (V.D.R.) as described by Burba *et al.* (1983).

After 240 days of storage sensory evaluation was carried out by a ten-judge taste panel which was asked to rate the acceptability of raw garlic for human consumption.

RESULTS AND DISCUSSION

Irradiated garlic bulbs lost less weight than control ones after 150 days post-harvest (Fig. 1). At the end of the storage period the weight losses were 43% and 22% of the original weight in control and irradiated garlic bulbs, respectively. The weight loss reduction of irradiated bulbs has been attributed to the absence of sprouting and the inhibitory effect of radiation on both certain oxidases (El-Oksh *et al.* 1971) and respiratory activity (Michiels 1967). Recent biochemical and morphophysiological studies indicate that gamma rays accelerate inner sprout leaf senescence and delay the storage leaf senescence of garlic seed cloves (Croci 1988). For garlic bulbs the majority of the weight consists of the storage leaf weight. Thus for irradiated garlic bulbs the delayed senescence of storage leaf could be the cause of weight loss reduction.

In support of these facts a decreased growth of inner sprout leaf in terms of V.D.R. in irradiated seed cloves was observed (Fig. 4). This result is in line with the plastochronic rhythm arrest induced by irradiation in the garlic clove meristem (Croci 1988).

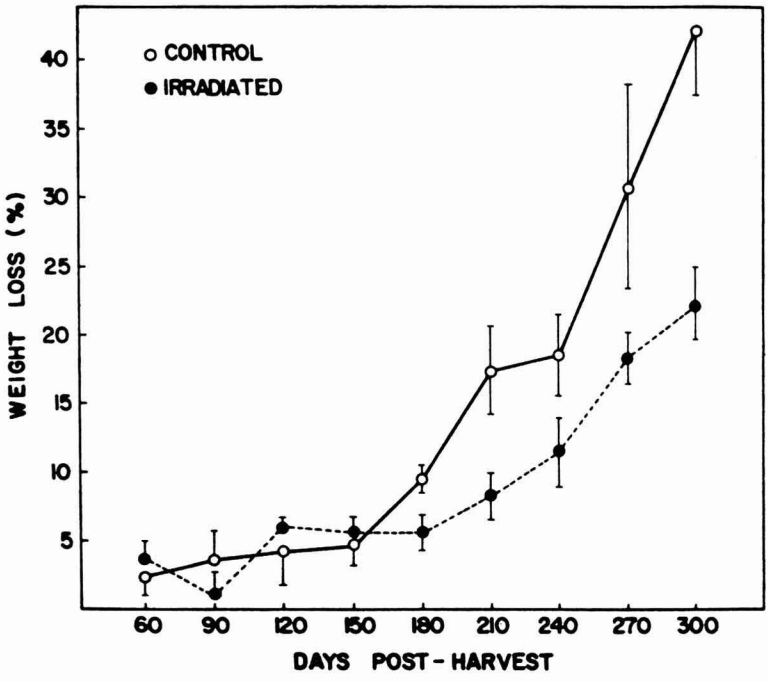

FIG. 1. LOSS OF WEIGHT IN GARLIC BULBS STORED AT 6–32°C (R.H. 40–50%) UP TO 300 DAYS

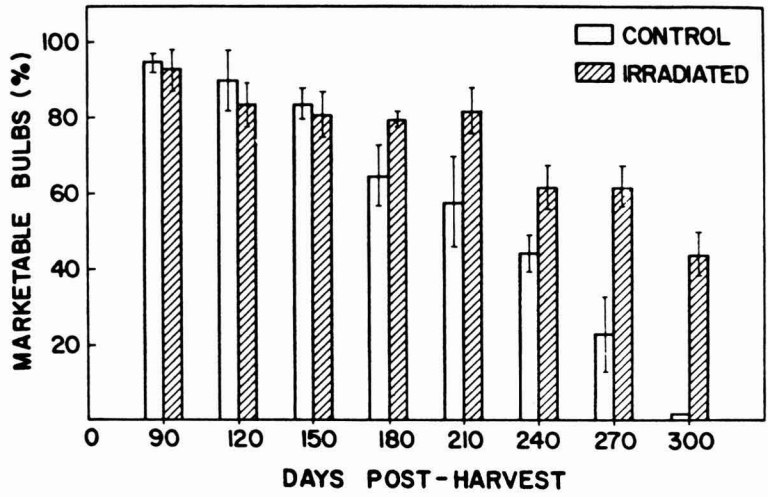

FIG. 2. PERCENTAGE OF MARKETABLE GARLIC BULBS STORED AT 6–32°C (R.H. 40–50%) UP TO 300 DAYS

Data presented in Fig. 2 and 3 show that irradiation improved the percentage of marketable bulbs and marketable cloves after 150 days postharvest. Control bulbs and cloves were discarded due to sprouting and rotting; irradiated bulbs and cloves were discarded only due to rotting. At the end of the storage period (300 days after harvest) the irradiated sample had 42% more marketable bulbs than the control. For the same storage period a minor profit (33%) of marketable bulbs was reported on irradiated Colorado garlic (a late cultivar) by Croci and Curzio (1983). This suggests that the radioinhibition process applied to an early cultivar offers more benefit than to a late one.

Sensory evaluation revealed no adverse effects on the organoleptic properties of irradiated garlic at the end of the storage period.

In conclusion, the garlic cultivar selected had a satisfactory behavior in response to treatment since this decreased the weight loss and extended the storage life of the bulbs. These results clearly show that the "Rosado Paraguayo" garlic cultivar is suitable for irradiation treatment for raw/fresh marketing. Also, on the basis that sprouting is a disadvantage in bulb processing (Thomas 1984), the radioinhibition process is a promising tool for the garlic processing industry.

ACKNOWLEDGMENTS

Thanks are given to the Comisión Nacional de Energía Atómica for irradiation processing. Thanks are also given to Ms. Ana M. Urioste for the sensory evaluations and to Mr. Walter H. Galié for the technical assistance.

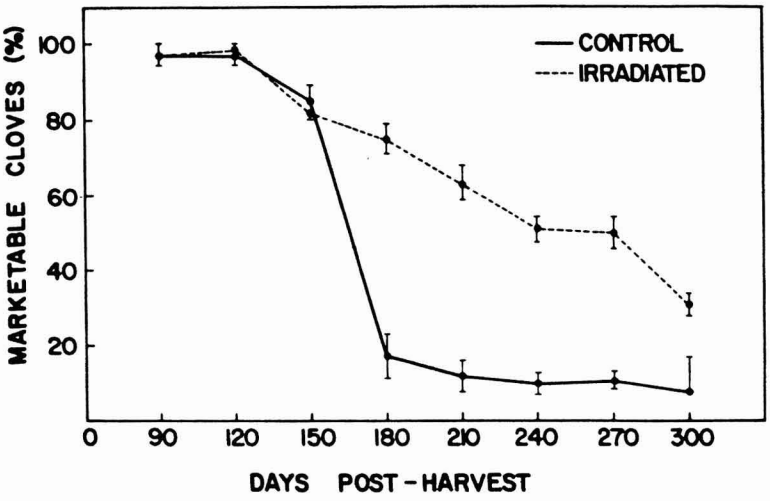

FIG. 3. PERCENTAGE OF MARKETABLE GARLIC CLOVES STORED AT 6–32°C (R.H. 40–50%) UP TO 300 DAYS

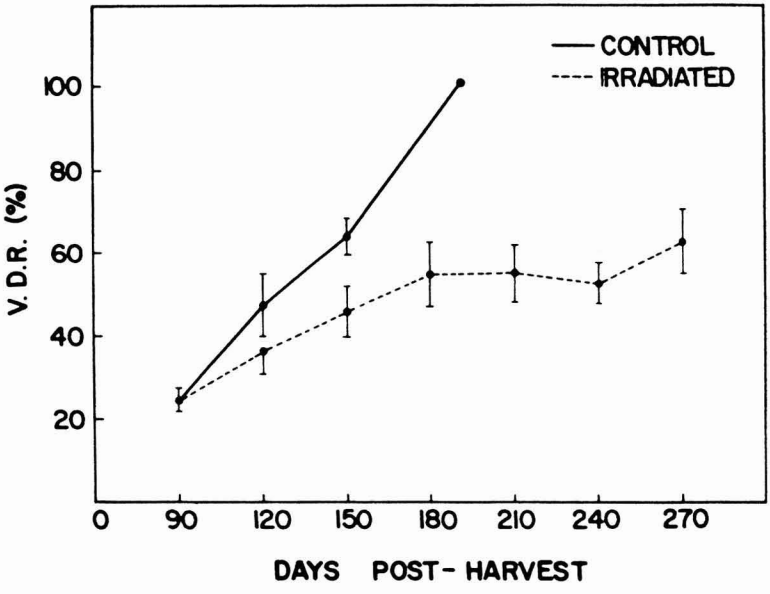

FIG. 4. VISUAL DORMANCY RATE OF GARLIC CLOVES STORED AT 6–32°C (R.H. 40–50%) UP TO 270 DAYS

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SPROUT INHIBITION BY GAMMA IRRADIATION IN FRESH GINGER (*ZINGIBER OFFICINALE* ROSCOE)

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Accepted for Publication June 7, 1989

ABSTRACT

A study on sprout inhibition by gamma irradiation in fresh ginger of a local variety was carried out. Fresh ginger was irradiated at the doses of 0, 25, 50 and 80 Grays (Gy) and stored at temperature 25–28°C and relative humidity ranging from 76–96% for 4 months. The parameters observed were physiological weight loss, sprouting, external appearance, fungal infection, moisture content, water activity, crude fiber content and total sugar. The results show that irradiation at the doses studied effectively inhibited sprouting in ginger when compared to the nonirradiated samples. However, radiation was unable to extend the shelf-life as all samples started to deteriorate after 2 months storage.

INTRODUCTION

Among the various applications of ionizing radiation in the preservation of horticulture products, shelf-life extension of onions and potatoes by inhibition of sprouting has been successfully practiced commercially in several countries such as Japan and East Germany (Anon. 1987). Laboratory scale studies on garlic, chestnut and ginger have also shown the feasibility of using irradiation to inhibit sprouting. Irradiation treatment appears to be justified in terms of reduced postharvest loss and improved quality products (Moy 1973).

More ginger is being grown in Malaysia to meet the increasing demand from local and export markets. There is a growing interest in preserving the product by sprout inhibition and shelf-life extension. The shelf-life of fresh ginger is often terminated by sprouting, moldiness, shrivelling and softening. Of these factors, sprouting is the most obvious manifestation of deterioration. At present, low temperature treatment (10–15°C) has been recommended for storing fresh ginger up to 4 months without sprouting (Abd. Shukor *et al.* 1986). However,

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Journal of Food Processing and Preservation **14** (1990) 113-122.

the low temperature storage technique is rather expensive under our tropical climate or during transportation.

A study was undertaken to identify effective irradiation doses which can inhibit sprouting in a Malaysian local variety of fresh ginger (*Zingiber officinale* Roscoe) during storage under ambient conditions. The effects of radiation on physical and chemical properties and other parameters that determine the keeping quality of fresh ginger are also reported.

MATERIALS AND METHODS

Freshly harvested ginger (variety: Lowland) of 8–9 months after planting was purchased from the nearby Federation Agricultural Marketing Authority (FAMA). The ginger was sorted, washed and dipped in benomyl suspension (500 μg/mL) for 3 min as described by Abd. Shukor *et al.* (1986). The pre-treatment was necessary to reduce fungal infection during storage. Curing was then carried out by allowing the ginger to remain at room temperature for 4 to 5 days.

Irradiation was carried out in Gamma Cell G.C 4000A, cobalt 60 source, with a dose rate of 101.46 Gy/min at average doses of 0, 25, 50 and 80 Gy. The average dose uniformity of 1.50 was obtained in the samples at density 0.933. The irradiated ginger was placed in perforated plastic trays and stored at room temperature (25–28°C) and humidity ranging from 76 to 96% for 4 months. Each tray which represented a replicate contained 1.5 kg of ginger and each treatment was done in 3 replicates.

All samples were checked monthly for physiological weight loss, external appearance, sprouting and fungal infection. An arbitrary scale ranging from 1 to 5 was designed for the rating. The pound samples were analyzed for moisture content determination using oven-drying method (Abd. Shukor *et al.* 1986), crude fiber content by AOAC method (1970), water activity estimation using Humidat-IC11 (Muri 1984) and total sugar assay using DNS method (Miller 1959) at 0, 1, 3 and 4 months of storage. Data were analyzed statistically by analysis of variance.

RESULTS AND DISCUSSION

Physiological Weight Loss

A significant decrease ($P < 0.001$) in fresh weight was observed during storage in all samples. After the second month of storage, about 50% weight loss was recorded in all treatments (Fig. 1). The loss in weight is believed to be mainly due to respiration and/or transpiration (Abd. Shukor *et al.* 1986). The irradiated ginger samples showed a significantly higher weight loss ($P = 0.05$) than the

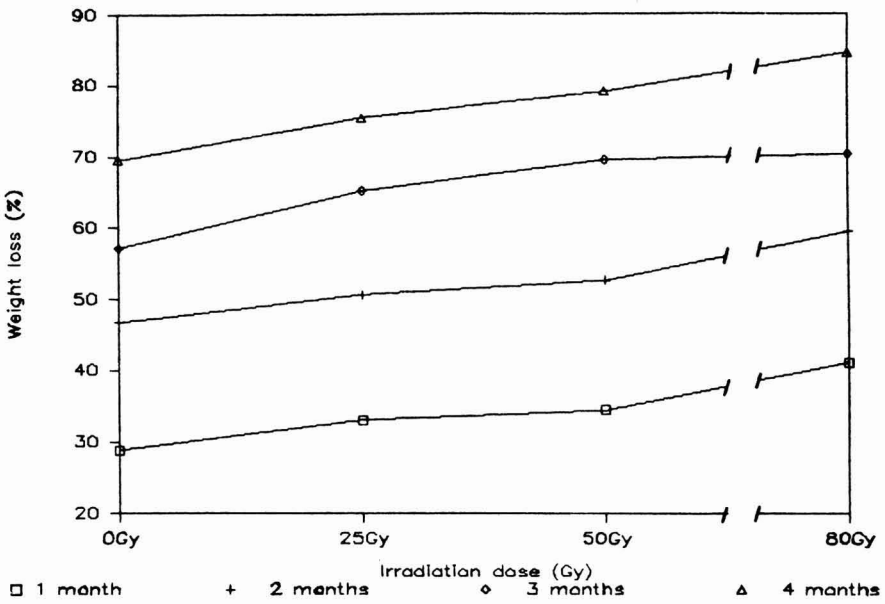

FIG. 1. EFFECT OF RADIATION AND STORAGE ON WEIGHT LOSS (%) OF GINGER UNDER AMBIENT CONDITIONS

nonirradiated ginger after 1, 2 and 4 months' storage. A study carried out in Thailand reported a similar observation when irradiated at 40 to 100Gy and stored for 6 months at 20 ± 1°C and relative humidity of 75%; however the effect of irradiation was not significant at 32 ± 3°C when a great extent of weight loss was observed in all samples (Sirikulvadhana and Prompubesara 1979).

The results also indicate that irradiation may have an immediate effect on respiration which results in significant reduction of weight in ginger. Increase in the respiration rate as an immediate effect of ionizing radiation has been well documented in potatoes (Thomas 1984). Early studies on respiratory metabolism of potatoes irradiated at low doses for sprout inhibition suggested that the increase in respiration was attributed to an increased oxidative phosphorylation. The inability of the irradiated tubers to heal a normal skin damage caused by harvesting and handling, as claimed by these workers, may hamper the protection against water loss by evaporation. Figure 1 shows that increase in radiation dose resulted in proportional increase in weight loss at all storage periods.

Sprouting

As shown in Table 1, more than 50% sprouting was only observed in the nonirradiated ginger at 1 month storage. Although <10% sprouting was observed

TABLE 1.
SPROUTING OF GINGER STORED IN AMBIENT FOR 4 MONTHS

Irradiation dose (Gy)	Storage period (months) 0	1*	2	3	4
Control	1	5 a	5 a	5 a	5 a
25	1	2 b	1.7 b	1 b	1 b
50	1	1.7 b	1 c	1 b	1 b
80	1	2 b	1 c	1 b	1 b

+
Rating : 1=no sprouting,
2=less than 10% sprouting,
3=10 to 25% sprouting,
4=25 to 50% sprouting,
5=more than 50% sprouting.

* Means which are not significantly different are followed by the same letter (sig. level = 0.05).

in irradiated samples during the first month, the growth was inhibited in the following months. The results suggest that radiation may have disrupted the cell division and multiplication which apparently occurs actively in the bud tissues. The effect was slower at lower dose (25Gy). Previous work in Hawaii has suggested that sprouting could be controlled even at a much lower dose of 10 Gy (Moy 1973) while a combined treatment of 40 Gy with cool temperature has been recommended by Sirikulvadhana and Prompubesara (1979).

External Appearance

The external appearance of ginger samples in all treatments showed symptoms of deterioration after one month of storage. More than half of the samples became unacceptable after 2 months as a result of shrinkage and discoloration (Table 2). The average shelf-life of fresh ginger when kept under ambient conditions was found to be less than 2 months. Abd. Shukor *et al.* (1986) reported earlier that ginger stored under ambient conditions would remain acceptable up to 1 month. The considerable weight loss recorded in the irradiated ginger did not contribute to the external appearance.

Fungal Infection

No fungal infection was observed in the control until the fourth month of storage. For the samples irradiated at 50 and 80 Gy, slight fungal infection could

TABLE 2.
EXTERNAL APPEARANCES OF GINGER STORED IN AMBIENT FOR 4 MONTHS

Irradiation dose (Gy)	Storage period (months) 0	1*	2	3	4
Control	1	3 ab	5	5	5
25	1	2.7 b	5	5	5
50	1	2.7 b	5	5	5
80	1	4.2 a	5	5	5

+
Rating : 1=100% as initial samples,
2=90% as initial samples,
3=75 to 90% as initial samples,
4=50 to 75% as initial samples,
5=less than 50% as initial samples.

* Means which are not significantly different are followed by the same letter (sig. level = 0.05).

be observed after 3 months. All irradiated samples became significantly infected ($P = 0.05$) at the end of the study (Table 3). This observation suggests that the radiation treatment could account for the susceptability of the stored ginger to infection or rotting. There is plenty of evidence that irradiated potatoes are more susceptible to rotting if the wounds induced during handling have not been allowed to heal before irradiation occurs (Thomas 1984). With careful handling before and after irradiation, infection and rotting could be avoided in ginger rhizomes.

Moisture Content

The effect of radiation and storage on the moisture content of the ginger was identified by monitoring the changes in the moisture content itself. The average moisture content of all fresh samples was about 85.6% and this value was found to gradually decrease with storage time (Fig. 2). There was no significant difference in moisture content between the control and irradiated samples during the first three months. However, in the fourth month, a significantly low moisture content was observed in ginger samples irradiated at 80 Gy which had also recorded the highest weight loss ($P = 0.05$). Ginger samples irradiated at 25 and 50 Gy showed only a slight decrease in the moisture content though they had a significant weight loss when compared to the control.

TABLE 3.
FUNGAL INFECTION IN GINGER DURING STORAGE IN AMBIENT FOR 4 MONTHS

Irradiation dose (Gy)	Storage period (months) 0	1	2	3	4*
Control	1	1	1	1	1 b
25	1	1	1	1	3 a
50	1	1	1	3	3 a
80	1	1	1	2	3.7 a

+
Rating : 1=0% infection,
2=less than 5% infection,
3=5 to 25% infection,
4=25 to 50% infection,
5=more than 50% infection.

* Means which are not significantly different are followed by the same letter (sig. level = 0.05).

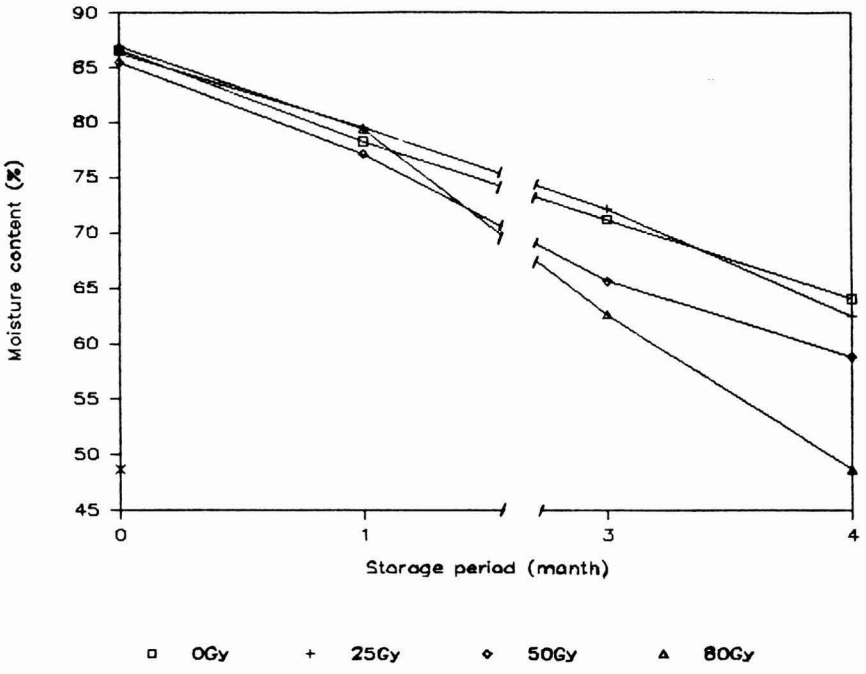

FIG. 2. EFFECT OF RADIATION AND STORAGE ON MOISTURE CONTENT (%) OF GINGER UNDER AMBIENT CONDITIONS

Crude Fiber Content

The changes in crude fiber content in all the samples showed that there was no significant effect due to storage and radiation dose although a steady increase in crude fiber content was observed after 1 month of storage (Fig. 3). The average value of 1.73% was estimated initially for the fresh ginger and this increased to 2.49% at the end of the study. The slight increase of crude fibre content during storage suggests that the ginger rhizomes may have become more fibrous (Abd. Shukor *et al.* 1986).

Water Activity

The average water activity or a_w value of 0.964 was recorded by all fresh samples and the level remained relatively constant within the range of 0.904–0.975 throughout the storage period (Fig. 4). The high value of a_w suggested that the soft tissues of fresh ginger were highly susceptible to bacterial growth and fungal infection throughout the storage period.

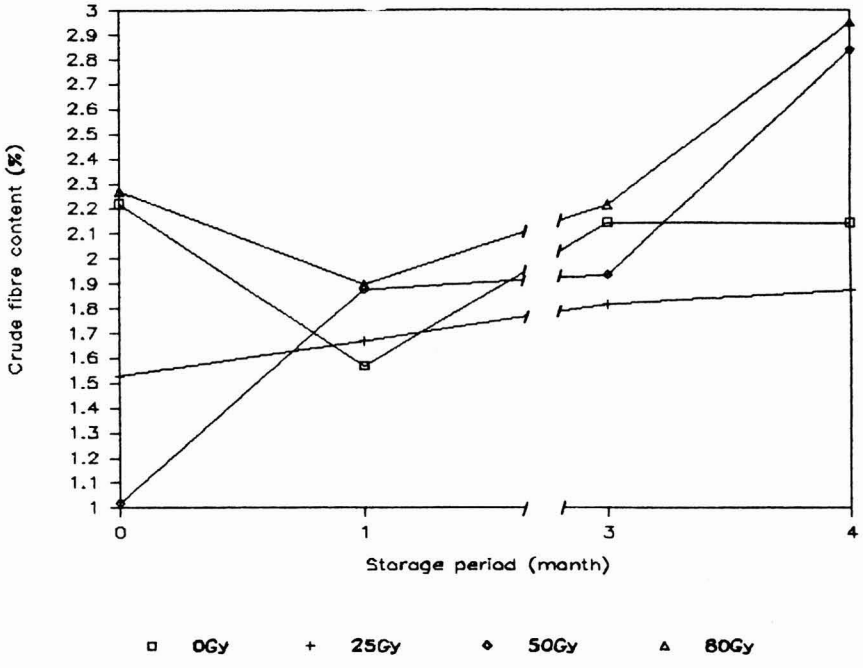

FIG. 3. EFFECT OF RADIATION AND STORAGE ON CRUDE FIBRE CONTENT (%) OF GINGER UNDER AMBIENT CONDITIONS

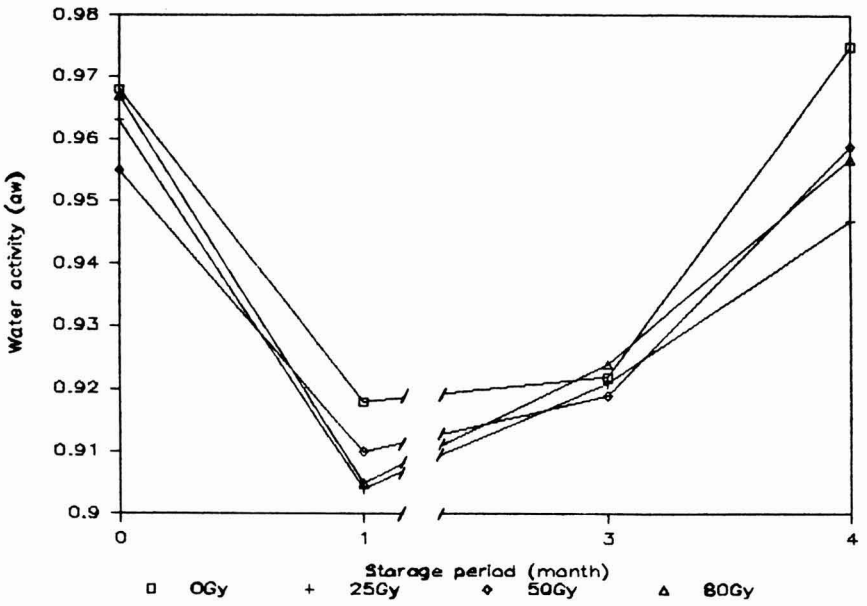

FIG. 4. CHANGES IN WATER ACTIVITY OF IRRADIATED GINGER DURING STORAGE UNDER AMBIENT CONDITIONS

Total Sugar

Changes in total sugar indicate changes in the amount of reducing sugars and sucrose. Figure 5 shows an increase in total sugar (%) immediately after irradiation, although not significantly, at all dose levels. The increase in total sugar immediately after irradiation suggests that hydrolysis of starch might have taken place which resulted in an increase in the reducing sugars. A significant decrease in total sugar ($P < 0.01$) in all samples after 1 month under storage may indicate that higher metabolic activities like respiration, where sugars are being consumed and converted into carbon dioxide and water, were in progress. In the control, this process was accompanied by elongation of the sprouts as described by Abd. Shukor *et al.* (1986). An increase in total sugar in ginger following irradiation agrees with many findings in irradiated potatoes where the increase was always accompanied by a decomposition of starch (Wills 1965). The occurrence of significant weight loss in irradiated ginger as reported earlier was presumably due to active respiration, rather than dehydration. Radiation has been shown to have no effect on moisture content when compared to non irradiated ginger during storage.

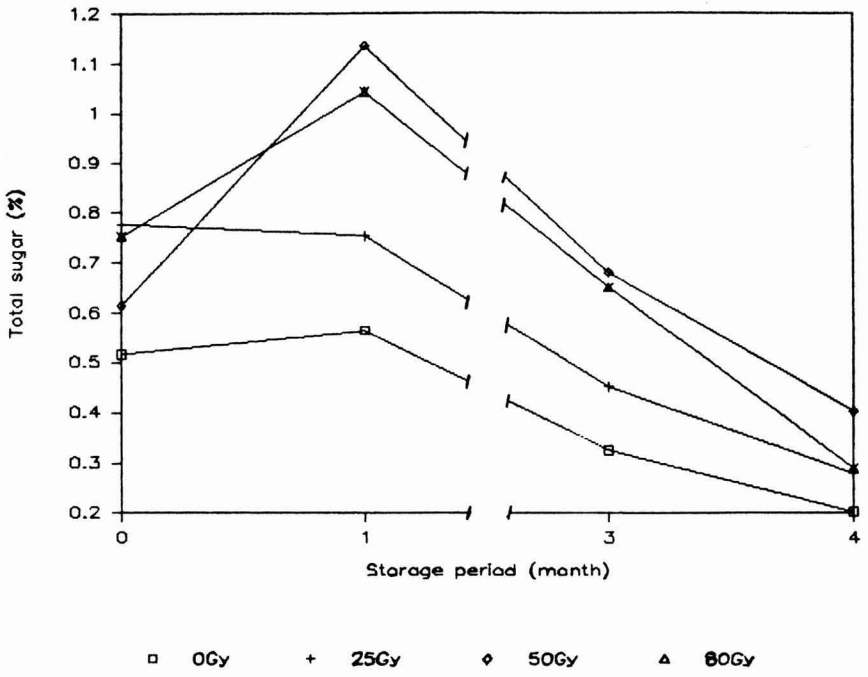

FIG. 5. EFFECT OF RADIATION AND STORAGE ON TOTAL SUGAR (%) OF GINGER UNDER AMBIENT CONDITIONS

CONCLUSION

This study shows that fresh ginger can be irradiated at as low as 25 Gy to inhibit sprouting when stored under ambient conditions. A dose of 80 Gy affected the external appearance after 1 month storage. Fungal infection was only apparent after 3 months of storage in both 50 and 80 Gy samples, while all irradiated samples were infected by the fourth month. The findings suggest that irradiation may be used to inhibit sprouting but is unable to extend the shelf-life of fresh ginger. Both irradiated and nonirradiated samples showed visual symptoms of losing keeping quality after 2 months under ambient conditions. Irradiation may therefore replace low temperature treatment (10–15°C) for sprout inhibition treatment, but other treatment is still needed for shelf-life extension. The present work proposes that any further studies should take into consideration a combined treatment of radiation and other methods of preservation such as cool temperature (20–25°C), good ventilated storage place or packaging which may prevent moisture loss. A combined treatment is believed to be able to inhibit sprouting as well as to extend the shelf-life of fresh ginger.

ACKNOWLEDGMENTS

The author would like to thank Ms. P. A. Wills, ANSTO, Australia and Mr. Abd. Shukor Abd. Rahman, MARDI, Malaysia for their critical reading of the article and useful discussions; Ms. Yasmin Yaacob, Mrs. Latifah Amin and Mrs. Salahbiah Abd. Majid for the technical assistance. The work was funded by the Nuclear Energy Unit, Malaysia (JKP/PP(1.3)/87).

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EFFECT OF WATER ACTIVITY ON VITAMIN A DEGRADATION IN WHEAT FLOUR (ATTA)

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Accepted for Publication June 7, 1989

ABSTRACT

The effect of water activity (a_w) on the stability of vitamin A palmitate adsorbed onto wheat flour, starch, gluten, enzyme inactivated wheat flour and microcrystalline cellulose was investigated. In wheat flour the rate of vitamin A degradation was lowest at 0.0 a_w. In gluten, starch and enzyme inactivated wheat flour, the rate was highest at 0.0 a_w and decreased with rise in a_w. The rate of lipid oxidation in wheat flour was lowest at 0.0 a_w whereas in enzyme inactivated wheat flour it was highest at 0.0 a_w and decreased with rise in a_w. In microcrystalline cellulose system vitamin A degradation was least at 0.33 a_w and the rate of degradation increased both below and above this a_w. Among the wheat flour constituents, gluten provided better protection to vitamin A than starch. The role of lipoxygenase enzyme in accelerating the rate of degradation of vitamin A in wheat flour at higher a_w has been suggested.

INTRODUCTION

Degradation of nutrients especially vitamins during storage poses major problems in the fortification of foods for nutritional supplementation programs. Vitamin A is highly prone to oxidation in the presence of oxygen and its degradation is associated with the development of off-flavors. Its degradation therefore not only affects the nutritional value but also the sensory quality and acceptance of stored products. In foods vitamin A undergoes cooxidation with lipids and the rate of oxidation is influenced by the water activity (a_w). The rate of autoxidation of lipids is generally minimal at monolayer a_w and both below and above this value the reaction proceeds significantly faster (Chou *et al.* 1973). Arya and Parihar (1981) and Arya (1981) have, however, observed that in whole wheat flour and bengalgram flour, carotenoids degraded much more slowly at 0.0 a_w than at 0.73 a_w. Earlier Cuendet *et al.* (1954) had also reported that stability of

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wheat flour decreased when moisture content was increased from 3 to 14%. Detrimental effects of higher moisture on Vitamin A stability in vitamin enriched wheat flour has been reported by Feller and Bean (1977) and Cort *et al.* (1976). During processing of dehydrated and preserved foods, the raw materials are generally blanched to inactivate enzymes. But wheat flour contains active enzymes and these may have a role in determining the effect of a_w on rates of vitamin A degradation during storage. The role of lipoxygenase in the cooxidation of carotenoids and vitamin A has been reported previously (Davies 1979; Barimalaa and Gordon 1988). The present study was designed to determine the effect of a_w on the stability of vitamin A when adsorbed onto wheat flour, enzyme inactivated wheat flour, cellulose, gluten and starch in isolated model systems to understand the role of enzymes and major wheat flour constituents in vitamin A degradation at different a_w.

MATERIALS AND METHODS

All solvents were of GR grade (E. Merck) and were used as such without further purification. Retinyl palmitate (vitamin A) was from Sigma Chemicals, USA and microcrystalline cellulose (Avicel) from FMC Corporation, Marcus Hook, Pa 19061.

Wheat Flour

Sharbati wheat (Bhojan Samrat) was ground in a commercial flour mill and whole wheat meal was shifted through a 30-mesh sieve. The proportion of bran and flour (atta) were 10% and 90%, respectively.

Gluten and Starch

Wheat flour (500 g) was treated with water (300 mL) and the contents were mixed by hand in a stainless steel dish to form a firm dough. After wrapping in muslin cloth, the dough was left standing in water for one hour. To separate gluten and starch, the dough was kneaded gently under a stream of water over a fine muslin cloth and the washings were collected in a 2 L beaker. After complete washing of starch and water solubles, the gluten was removed from muslin cloth and allowed to stand in distilled water in a beaker and again kneaded and washed thoroughly. The gluten was pressed in palms to remove excess water and dispersed in 250 mL chloroform-methanol (2:1) mixture in an electric blender, filtered through a sintered funnel, dried in an air oven at 100°C and powdered.

The washings in the beaker were allowed to settle and excess water was removed by decantation. The starch was resuspended in distilled water, allowed

to settle and excess water decanted. The starch slurry was centrifuged and after decanting the supernatant, the residue was resuspended in 500 mL chloroform-methanol (2:1) to remove lipids. The slurry was filtered through a sintered funnel and the starch was dried in an air oven at 100°C. The dried starch was ground in a glass mortar.

Enzyme Inactivated Wheat Flour (atta)

One hundred g samples were heated in petri dishes (15 cm dia) at 100°C for 2 h in an air oven. In another experiment 100 g samples were treated with 100 mL methanol. After 4 h the methanol was evaporated under vacuum in a rotary thin film evaporator (Evapotec ™). Both these treatments were found to completely inactivate lipase and lipoxidase activity in the flour.

Model System Development

Retinyl palmitate (200 mg) was dissolved in chloroform (50 mL) and was quantitatively added to 80 g adsorbents (microcrystalline cellulose, starch, gluten or wheat flour) in a 1-L RB flask. The flask was swirled to form a homogenous slurry and the chloroform was evaporated under vacuum in a rotary thin film evaporator (Evapotec ™). Retinyl palmitate treated adsorbents were ground in a glass mortar to break lumps and redried under vacuum till completely freed from solvent. The treated adsorbents (15 g) were stored in petri dishes (15 cm dia) in desiccators containing phosphorus pentoxide and saturated solutions of magnesium chloride and sodium nitrate, respectively, at room temperature (15–35°C) in dark.

Analysis

After moisture equilibration and during storage 100 mg samples were treated with 10 mL aliquots of chloroform and filtered through a sintered funnel. The residue was extracted three times with 10 mL aliquots of chloroform and filtered. The combined filtrate was made to 50 mL with chloroform and its absorbance was measured at 325 nm using Shimadzu UV 240 Graphicord spectrophotometer. The moisture content was determined by heating 2 g samples in tared aluminium dishes (8 cm dia.) at 100 $\pm$2°C for 4 h. TBA value was determined by the method of Tarledgies *et al.* (1960) and fat acidity by AACC method (1957).

Lipoxygenase Activity

Lipoxygenase activity in wheat flour was measured by the method of Surrey (1964). Wheat flour (5 g) was extracted with cold (3–5°C) 50 mL, 0.05 M phosphate buffer, pH 6.0 in an ice bath for 1 h, swirling the mixture for 1 min

every 15 min. The mixture was centrifuged at 15,000 × g for 15 min at 4°C. Lipoxygenase activity was determined by measuring the increase in absorbance at 234 nm at 25°C of the reaction mixture consisting of 2.90 mL of 0.05 M phosphate buffer, pH 6.0, 0.02 mL of the enzyme extract and 0.02 mL of linoleic acid substrate (8.05×10^{-3}M) prepared in borate buffer, pH 9.0.

For preparing substrate, linoleic acid (0.25 mL) was added dropwise to a solution of 5.0 mL of 0.05 M borate buffer, pH 9.0 containing Tween-20 (0.25 mL) with continuous stirring. The resultant suspension was clarified by adding 0.65 mL of 1 N sodium hydroxide. The volume was adjusted to 50 mL with additional borate buffer and finally to 100 mL with distilled water.

No change in the absorbance of the reaction mixture for 2 min was considered complete inactivation of the lipoxygenase activity.

Lipase Activity

Lipase activity was measured by the method of Fiore and Nord (1949).

RESULTS AND DISCUSSION

Effect of a_w on the rates of degradation of retinyl palmitate when adsorbed onto microcrystalline cellulose and wheat flour is shown in Fig. 1. It may be observed that in wheat flour the rates of degradation of retinyl palmitate is highest at 0.73 a_w and decreases with the decrease in a_w. After 70 days of storage percentage retention of retinyl palmitate was 74, 68 and 59, respectively, at 0.0, 0.33 and 0.73 a_w which is in conformity with the results reported earlier (Arya and Parihar 1981; Arya 1981; Feller and Bean 1977 and Cort *et al.* 1976). On the other hand, in microcrystalline cellulose system the rate of degradation was least at 0.33 a_w. Since monolayer a_w in microcrystalline cellulose system is around 0.33, highest retention at this a_w is in conformity with the published information (Labuza *et al.* 1969).

In order to understand the role of wheat flour constituents on vitamin A degradation, retinyl palmitate was adsorbed onto starch and gluten and stored at different a_w. The rates of vitamin A degradation are shown in Fig. 2. It is interesting to observe that in both starch and gluten systems the rates of vitamin A degradation were highest at 0.0 a_w. Also, in both the systems the rate of vitamin A degradation decreased with rise in a_w. After 70 days storage retention of retinyl palmitate was 57.9, 78.4 and 82.4% in gluten system as compared to 10.0, 40.0 and 48.5% retention in starch system at 0.0, 0.33 and 0.73 a_w. The same pattern has been observed earlier by Arya *et al.* (1979), Premavalli and Arya (1985) and Ramakrishnan and Francis (1979) for β-carotene degradation in isolated model systems. On the other hand, Chou *et al.* (1973) and Labuza

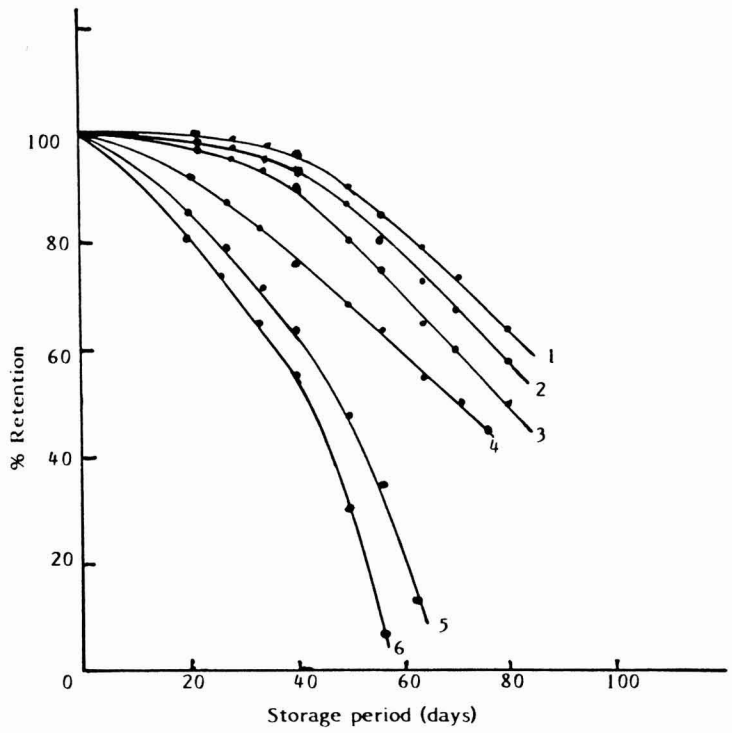

FIG. 1. EFFECT OF WATER ACTIVITY ON STORAGE STABILITY OF RETINYL PALMITATE IMPREGNATED ON WHEAT FLOUR AND MICRO-CRYSTALLINE CELLULOSE
1,2,3—Wheat flour at 0.0, 0.33 and 0.73 a_w, respectively;
4,5,6—Micro-crystalline cellulose at 0.33, 0.73 and 0.0 a_w, respectively.

et al. (1970) have reported that rate of lipid peroxidation is minimum at monolayer a_w and both below and above this a_w the rates of lipid oxidation were found to increase. The rise in the rate of lipid oxidation above monolayer a_w had been attributed to the increased diffusion of transition metal ions at higher a_w which catalyse lipid oxidation and as a result of increased swelling of the adsorbent matrix which helps in faster absorption of oxygen. In the present study the transition metal ions were not included and therefore the increase in the rate of vitamin A degradation as a result of increased mobilization of metal catalysts at higher a_w may not be appreciable. The inhibitory effect of water therefore seems to have continued even above the monolayer value in both wheat gluten and starch systems.

It is interesting to observe that though the pattern of vitamin A degradation with rise in a_w was the same both in gluten and starch systems, relatively the rate of degradation was considerably higher in wheat starch than in gluten system. Earlier, Ramakrishnan and Francis (1979) and Premavalli and Arya (1985) have

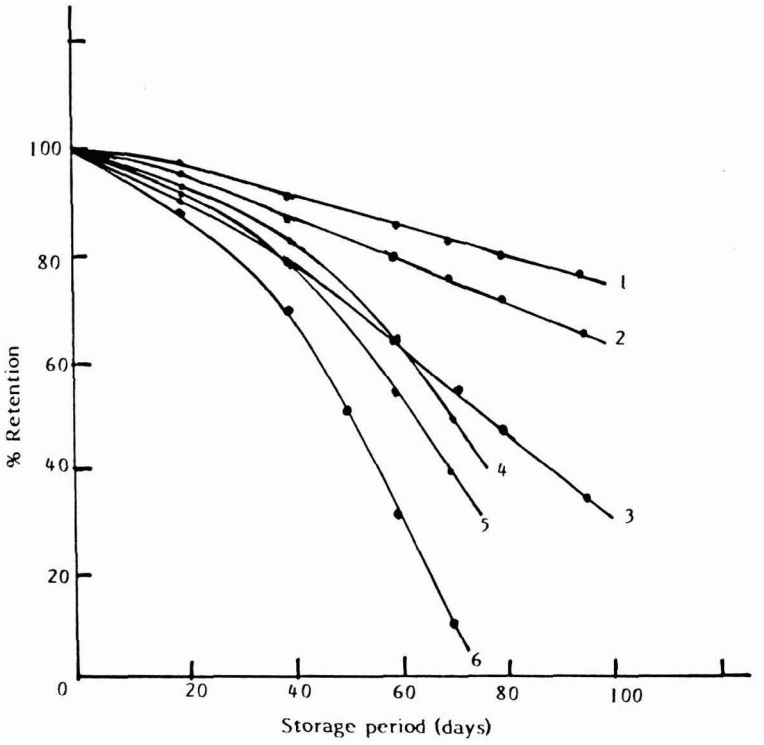

FIG. 2. EFFECT OF WATER ACTIVITY ON STORAGE STABILITY OF RETINYL PALMITATE IMPREGNATED ON GLUTEN AND STARCH
1,2,3—Gluten and 4,5,6—Starch samples at 0.73, 0.33 and 0.0 a_w, respectively.

also reported significant effect of adsorbents on the rates of degradation of carotenoids in model systems. Ramakrishnan and Francis (1979) have attributed the increased stability of carotenoids in starch system to the higher moisture absorption capacity of starch than cellulose. The results of the present study, however, do not support the above conclusion. The equilibrium moisture contents of gluten were 2.50%, 6.0% and 9.8% and that of wheat starch were 4.2%, 8.5% and 13.0% at 0.0, 0.33 and 0.73 a_w, respectively. It is apparent that despite higher moisture absorption capacity of wheat starch than that of gluten, vitamin A degraded considerably faster in starch system than in gluten system. Previously, Premavalli and Arya (1985) also could not correlate the relative degradation rates of carotenoids to the moisture absorption capacities of the different adsorbents. Since the rates of Vitamin A degradation were considerably lower in gluten than in starch at each of the three a_w studied, it is probable that the higher stability of vitamin A in gluten system may have resulted from the phys-

icochemical interaction of vitamin A with gluten. A direct evidence of these interactions however remains to be demonstrated.

Both wheat and wheat flour are known to contain lipase and lipoxygenase enzymes (Zeleny 1954; Guss *et al.* 1968 and Mann and Morrison 1977) and these may have a role in the degradation of vitamin A and carotenoids as enzyme catalyzed reactions proceed faster at higher a_w (Acker 1969). The role of lipoxygenase enzyme in the cooxidation of vitamin A and carotenoids in the presence of linoleic acid is well known (Barimalaa and Gordon 1988; Davies 1982; McDonald 1979; Matsuo *et al.* 1970; Grosch, *et al.* 1976). In order to understand the role of enzymes in the degradation of vitamin A at different a_w, wheat flour was heated at 100°C for 2 h or treated with methanol and retinyl palmitate was adsorbed onto enzyme inactivated flours. The rates of vitamin A degradation in enzyme inactivated flours at different a_w are shown in Fig. 3. It is interesting to note that in enzyme inactivated flours the degradation of retinyl palmitate was

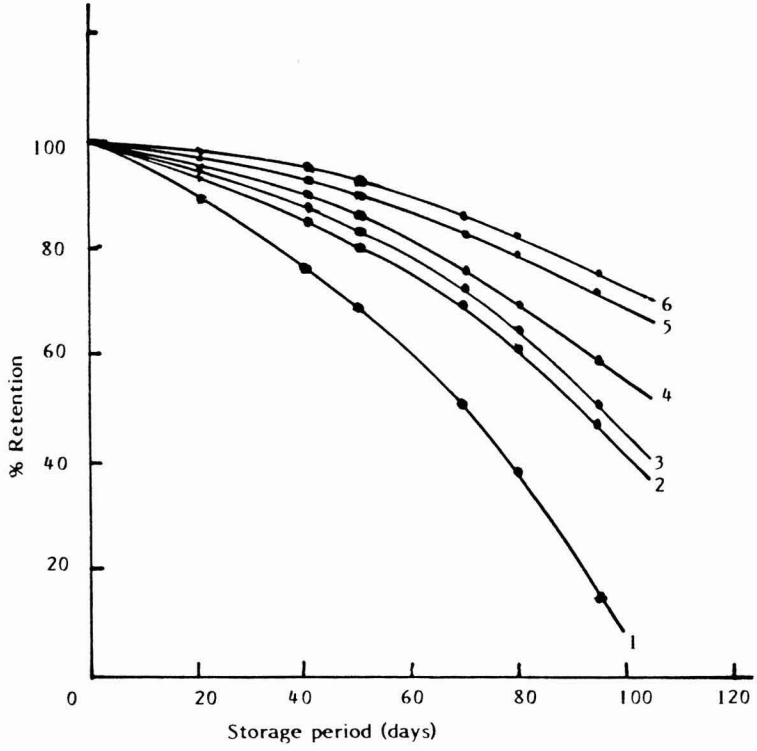

FIG. 3. EFFECT OF WATER ACTIVITY ON STORAGE STABILITY OF RETINYL PALMITATE IMPREGNATED ON ENZYME INACTIVATED WHEAT FLOUR
1,3,5—Heat inactivated wheat flour and 2,4,6—Methanol inactivated wheat flour stored at 0.0, 0.33 and 0.73 a_w, respectively.

highest at 0.0 a_w and the rate of degradation decreased with increase in a_w. The rate of degradation was lowest at 0.73 a_w as has been observed in gluten and starch systems. After 70 days storage retentions of retinyl palmitate were 52.2, 77.7 and 85.1% in enzyme inactivated flour as compared to 70.2, 64.2 and 55.9% retention in enzyme active flour at 0.0, 0.33 and 0.73 a_w, respectively.

Apparently at higher a_w enzymes in wheat flour seem to catalyse the degradation of retinyl palmitate. At low a_w (0.0 and 0.33) the action of lipase and lipoxygenase enzymes becomes limited on account of inadequate substrate mobility because the rate of enzyme catalyzed reactions in dry materials is governed by the rate at which the substrate diffuses to the enzyme. At 0.73 a_w the rates of diffusion of the substrates and reaction products are considerably higher and therefore catalytic actions of lipoxygenase and lipase enzymes become predominant at this a_w. Relatively higher degradation of retinyl palmitate in enzyme inactive flour than in enzyme active flour at 0.0 a_w (52.2% as compared to 70.2% retention after 70 days storage) may be atrributed to the destruction of natural antioxidants or due to formation of hydroperoxides as a result of lipid oxidation which occurs due to heating at 100°C. This is further supported from the fact that methyl alcohol inactivated flour retained almost the same level (72.5%) of retinyl palmitate as that of enzyme active flour (70.2%) at 0.0 a_w after 70 days storage.

Though role of lipases in the degradation of carotenoids and vitamin A has not been reported earlier, the increased rate of lipolysis may influence the oxidative degradation of the vitamin in two ways. Firstly, free fatty acids are known to autoxidize faster than their triglycerides (Graille *et al.* 1974; Badings 1960; Dahle 1965) and secondly, wheat lipoxygenases catalyze oxidation of carotenoids and vitamin A in the presence of only free polyunsaturated fatty acids and their monoglycerides and not triglycerides and diglycerides (Guss *et al.* 1968; Mann and Morrison 1974). An increase in lipolysis of wheat lipids is therefore expected to accelerate the oxidative degradation of vitamin A which has been found to be the case in untreated enzyme active wheat flour when stored at 0.73 a_w. Earlier Dahle (1965) has also reported the accelerating effect of free polyunsaturated fatty acids (chiefly linoleic acid) in the oxidation of semolina carotenoids. At lower a_w (0.0 and 0.33), the action of lipases and lipoxygenases become limited due to inadequate substrate mobility and therefore retention of vitamin A and carotenoids in enzyme active flours is higher at 0.0 a_w than at 0.73 a_w. In enzyme inactive flours vitamin A and carotenoid degradation proceeds by normal autoxidative pathway and therefore as expected the rate of degradation was highest at 0.0 a_w and a gradual decline with rise in a_w.

The changes in fat acidity and TBA value of wheat flour and heat inactivated flour which indicate the hydrolytic and oxidative degradation of wheat lipids (Table 1) also support the above conclusions. It may be observed that at 0.0 and

TABLE 1.
EFFECT OF WATER ACTIVITY ON THE FAT ACIDITY
AND TBA VALUE OF WHEAT FLOUR (ATTA)

Product	Water activity	Fat acidity (mg KOH/100 g flour)		TBA Value (mg malonaldehyde/kg flour)	
		0	70 days	0	70 days
Wheat flour	0.0	20.3 ± 2.3	25.2± 2.7	0.08 ± 0.02	0.35 ± 0.05
	0.33	20.3 ± 2.3	45.5 ± 3.8	0.08 ± 0.02	0.43 ± 0.06
	0.73	20.3 ± 2.3	115.6 ± 4.5	0.08 ± 0.02	1.03 ± 0.08
Heat inactivated flour	0.0	27.6 ± 2.6	31.2 ± 2.4	0.10 ± 0.02	0.56 ± 0.06
	0.33	27.6 ± 2.6	38.3 ± 2.8	0.10 ± 0.02	0.40 ± 0.03
	0.73	27.6 ± 2.6	41.2 ± 3.5	0.10 ± 0.02	0.29 ± 0.03
Methyl alcohol inactivated flour	0.0	19.5 ± 2.4	26.5 ± 2.3	0.08 ± 0.02	0.44 ± 0.04
	0.33	19.5 ± 2.4	29.3 ± 2.6	0.08 ± 0.02	0.35 ± 0.03
	0.73	19.5 ± 2.4	39.5 ± 3.2	0.08 ± 0.02	0.24 ± 0.03

0.33 a_w, there were only slight increases in fat acidity in both enzyme inactivated and untreated flours. At 0.73 a_w also fat acidity increased very slowly in enzyme inactivated flour as compared to steep increase in untreated flour indicating the rapid hydrolysis of wheat lipids through lipase activity at higher a_w (0.73). Changes in TBA value though not so extensive as lipolysis also followed a similar pattern.

From the foregoing discussion it is evident that relatively higher losses of vitamin A in wheat flour during storage at higher a_w is brought about by the action of lipase and lipoxygenase enzymes. At lower moisture levels the enzyme action becomes limited as a result of inadequate substrate mobility. Relatively vitamin A is more stable in gluten than in wheat starch matrix.

ACKNOWLEDGMENT

We wish to thank Dr. T. R. Sharma, Director, Defence Food Research Laboratory, Mysore, for his valuable suggestions during the course of study.

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CARBOHYDRATE UTILIZATION AND GROWTH KINETICS IN THE PRODUCTION OF YOGURT FROM SOYMILK PART I: EXPERIMENTAL METHODS[1]

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Accepted for Publication July 12, 1989

ABSTRACT

Growth and sugar utilization of Lactobacillus bulgaricus *and* Streptococcus thermophilus *were measured in soymilk. Total cell counts in the inoculum were made using the Breed smear. Correlations were developed between direct microscopic count and biomass dry weight for* Lactobacillus bulgaricus *and for* Streptococcus thermophilus. *A 1 g to 1 g ratio of the pure cultures was found to be superior to a 1:1 cell number ratio for the mixed culture inoculum. Carbohydrate substrate concentrations (sucrose and stachyose) were followed by high performance liquid chromatography. The methodologies for making these measurements on set cup style soymilk yogurts were developed and checked for precision in this study.*

INTRODUCTION

Utilization of soymilk as an ingredient for human food necessitates a reduction in the concentration of the carbohydrates (mainly stachyose) that can cause flatulence and a reduction in the objectional beany flavor typically associated with soy products. This work, part I, focuses on the key measurements made during soymilk fermentation. Part II (Buono 1988) describes the soy-yogurt fermentation by lactic acid bacteria. Part III (Buono 1988) considers the sensory

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Journal of Food Processing and Preservation **13** (1990) 135-153.

study of soy-yogurts by a trained panel. Part IV (Buono 1988) discusses and compares sensory measurements made by a trained panel and analytical measurements made by chemical instruments.

In order to carefully study soymilk fermentation, bacterial growth, product formation, and sugar consumption must be accurately measured. A proper ratio of rod:coccus in the inoculum is required. This ratio can be determined only by precise cell concentration measurements (Tamine and Deeth 1980). The amount of lactic acid produced during yogurt fermentation determines the endpoint of the fermentation. Many industrial yogurt making processes measure and use titratable acidity to set the point at which the yogurt is removed from the incubation room. Measurement of sugar consumption (especially stachyose) is also important in soy-yogurt production. By combining all of these measurements, it is possible to monitor the mass balance and estimate kinetic and yield parameters. These analytical results can be compared and correlated with sensory measurements.

THEORY

Liquid Chromatography

Normal phase high performance liquid chromatography (HPLC) conducted with an amine-based column can separate saccharides of different degrees of polymerization (i.e., mono-, di-, trisaccharides). The solution of sugars is carried by a polar liquid phase into the chromatography column for separation (Touchstone and Dobbins 1978). Amines, $-NH_2$, as a group possess an extremely electronegative nitrogen atom, which has a strong tendency to attract electrons. Nitrogen is also present in the mobile phase liquid mixture of acetonitrile, CH_3CN, and water, H_2O. This liquid mixture is commonly used in conjunction with an amine-based HPLC column for sugar separation (Morrison and Boyd 1973). Electronegative nitrogen will attract free electron pairs located in the outer shell of the oxygen atom. Hydroxyl groups in carbohydrates contain oxygen atoms, and thus, there is an interaction between nitrogen and oxygen from the mobile phase to the solid support and back to the mobile phase. Further, polar water in the mobile phase will also attract sugar hydroxyl groups back into the mobile phase from the solid support (Pomeranz 1985). Typically, reversible but strongest bonding occurs for the sugar that contains the largest number of hydroxyl groups. Separation of different sugars is realized by their residence time distributions on the solid support, which are determined by this dynamic equilibrium between support and mobile phase. The stereochemistry of the sugar molecules together with the number of binding sites on the molecule determine the amount of time before the sugar emerges from the column in the mobile phase.

Cell Concentration

Three methods for measurement of microbial growth are direct microscopic total cell counts (DMC), dry weight (dry wt or DW), and viable cell counts (VCC). Of utmost importance in yogurt making is that a 1:1 ratio of the rods:cocci be realized in the inoculum. Careful review of the literature (Tamine and Deeth 1980; Matalon and Sandine 1986) reveals that 1:1 ratio may refer to 1 cell to 1 cell or 1 colony forming unit to 1 colony forming unit or 1 g to 1 g. To achieve the desired ratio of rod cells to cocci cells the percent of the inoculum is fixed at 5% by volume, i.e.,

$$X + Y = (0.05) \times V \tag{1}$$

where:

X = volume of *Streptococcus* inoculum, mL
Y = volume of *Lactobacillus* inoculum, mL
V = volume of soymilk to be inoculated, mL

The second criterion is to determine the 1:1 ratio using either a colony forming unit, dry weight, or direct microscopic total cell count measurement. The descriptive equation is,

$$(A \times X) / (B \times Y) = 1.00 \tag{2}$$

where:

A = microscopic count or dry weight or colony forming unit count of *Streptococcus,* number/mL or g/mL or CFU/mL
B = microscopic count or dry weight or colony forming unit of *Lactobacillus,* number/mL or g/mL or CFU/mL

To measure a total count of cell number for rods and cocci, a Breed smear is made using a milk slide. The milk slide contains a printed 1.0 cm^2 circle onto which one loopful of milk sample is placed, approximately 0.01 mL. The organisms in the milk are counted after staining and the count of rods and cocci are converted into a concentration, C, as follows,

$$C = \text{Microscope Factor} \times \text{Microscope Count} \tag{3}$$

where

$$\text{Microscope Factor} = \frac{1.0\ cm^2}{\text{Area/Field}} / 0.01\ mL \tag{4}$$

where:

$1.0\ cm^2$ = area printed on the milk slide into which the milk sample was expressed.
Count = Microscopic Count = number of rods or cocci counted under the microscope.
0.01 = volume of liquid expressed onto the milk slide, mL
Area = area per microscope field, cm^2
C = cell concentration, cell number/mL

Area per field is calculated by measurement of the diameter of the microscope field, expressed in cm, using a micrometer viewed under oil immersion. The area is calculated from the diameter (D) by

$$\text{Area per field} = \pi D^2 / 4 \quad (5)$$

MATERIALS AND METHODS

The materials and methods are described fully by Buono (1988).

Soymilk Preparation

To prepare soymilk, 55.5 g of hand washed William's 82 soybeans were soaked for 14–16 h in 500 mL of distilled water at room temperature, 24.5°C, in a 1.0 L beaker. The next morning, the soak water was poured from the beaker, and the soybeans were transferred to a 1.0 L side arm flask. Tap water was added, and the beans were washed and stirred on an Amicon stir plate at a setting of 5.0 (or approximately 216 revolutions per minute) for approximately 10 to 15 min. The soybeans were placed in a Waring Futura Series blender, and 500 mL of boiling distilled water were added. The temperature of the water immediately after pouring was 85–90°C. The cap was quickly placed on the blender, and the controls were set at "blend" for ca. 3 min. The resulting aqueous mixture was filtered through two layers of 50 mesh Curity cheesecloth. Approximately 490 mL of soymilk were obtained per 55.5 g of soybeans. The flasks containing soymilk were capped with cotton plugs, covered with heavy duty aluminum foil and autoclaved at 121°C for 20 min. Total solids content of the soymilk was measured by drying 10 mL of soymilk at 105°C for 24 h (Buono 1988).

Starter Cultures and Culture Maintenance

Fresh starter cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were obtained from Microlife Technics in Sarasota, Florida. The organisms were received as pure cultures stored in 5 mL vials on 11% nonfat dry milk

(NFDM). A pure culture working stock of each organism was obtained by inoculating two 250 mL flasks, each containing 100 mL of an 11% NFDM solution, with a pure culture. Water and NFDM were mixed and autoclaved at 121°C for 15 min to prepare the 11% NFDM solution. The inoculated flasks were incubated overnight at 44°C until the milk coagulated. Sterile glycerol was added aseptically to each 250 mL flask to obtain 17% concentration by volume. The contents were mixed by shaking the flasks by hand for about 0.25 min and then aseptically transferred into 5 mL sterile vials. These vials were placed in an International Harvestor Model 70 freezer at −10°C. The glycerol in the milk served as a cryogenic agent to prevent freezing of the cultures and also served as a substrate source. The organisms were checked for purity by Gram's staining procedure, cell morphology, and a catalase test. Fresh inocula vials were prepared from the freezer stored vials at the first of each month. The past month's vials were discarded to prevent accidental use.

Preparation of Standard Curves

Inoculum studies were conducted to determine correlations relating total microscopic counts to dry weight, and total microscopic counts to viable cell counts. To eliminate interference from milk proteins in the dry weight analysis, these studies were conducted in Difco MRS broth for the *Lactobacillus* and in Difco APT broth for the *Streptococcus*. The inocula for these studies were prepared as described by Buono (1988). Forty-one mL of sample were taken every 30 min after the culture had been allowed to grow for four hours. Of the 41 mL sampled, duplicate 20 mL samples were used for dry weight measurements and 1 mL of sample was used for viable cell counts. Direct microscopic count was made using Breed smears on a milk slide (Tamine and Deeth 1980). *Lactobacillus bulgaricus* and *Streptococcus thermophilus* were enumerated separately by counting individual rods or coccus spheres, respectively, present in long growth chains. Cell counts were converted into counts per ml by use of the microscope factor discussed in the theory section.

The standard plate count method was used to evaluate viable cell count (Collins and Lyne 1984). The biomass cell dry weight in the fermenting broth was quantified using standard methods as described by Buono (1988).

Carbohydrate Analysis

Carbohydrates were extracted from the soymilk and prepared for injection into the HPLC using the method developed for bovine milk discussed by Kwak and Jeon (1986). Ten mL of milk sample were mixed with 15 mL of 2-propanol. The alcohol served as a denaturing agent and resulted in rapid precipitation of soymilk proteins. After exposure of the soymilk to 2-propanol for 20 min at

room temperature, the entire soymilk-alcohol mixture was centrifuged at 5,000 rpm for 15 min at room temperature, 24°C, in a Damon/IEC Division B-20A centrifuge. The supernatant was filtered in a 5.0 cm Buchner filter using Whatman #50 filter paper with a General Electric GEL 56110 vacuum pump. The filtrate was passed through Waters C-18 SEP-PAK filter cartridges to remove residual proteins, lipids, and chromophores. After prewetting (Buono 1988) and sample addition, 20 μl of the filtrate eluted from the SEP-PAK cartridges were injected directly into the HPLC and detected by refractive index. To permit back-calculation of the concentration of the carbohydrates in the 10 mL soymilk sample, the volume change on mixing was determined by measuring the total weight of the milk—alcohol mixture (Buono 1988).

To check the extraction recovery into the supernatant, the pellet from the centrifugation step was saved and analyzed for residual sugar. The pellet was first suspended into 15 mL of distilled water and mixed vigorously with a glass stir rod. The resulting slurry was centrifuged for 5 min at a setting of 5 in an International Clinical Centrifuge Model CL. The supernatant was filtered through a 5.0 cm piece of Whatman #50 filter paper by Buchner filtration. The filtrate was cleansed of nonpolar compounds by filtration through a prewet C-18 SEP-PAK cartridge. A sample of the eluting stream from the cartridge was injected into the HPLC, and the heights of the residual sugar peaks were measured.

To complete the check of the extraction procedure, recovery of sugar in solutions of known sugar content was studied. Six solutions of sucrose were prepared as test solutions: 10, 5, and 1 g of sucrose/L in aqueous solution and 10, 5, and 1 g sucrose/L in alcohol-water solutions. The ratio of alcohol to water in the latter three solutions matched the ratio of alcohol to water presented to the soymilk samples upon denaturation with 2-propanol; i.e., 60 mL of 2-propanol were added to 40 mL of aqueous solution. The aqueous-alcohol solutions were injected directly into the HPLC, and the sucrose peak from each was measured. The aqueous solutions were subjected to the extraction procedure of Kwak and Jeon (1986). The resulting streams eluting from the SEP-PAK cartridges for these three sugar solutions were also injected into the HPLC. The sucrose peaks from the extracted aqueous samples were evaluated. If the extraction method had indeed yielded complete recovery of the added sucrose into the effluent from the SEP-PAK filters, then the concentrations of sucrose in the water-alcohol preparations should have been identical to the concentrations of sucrose measured following the Kwak and Jeon extraction procedure.

The sample size injected into the HPLC was 20 μL. The HPLC used was a Varian Model number 5000 liquid chromatograph, with a LC-NH_2 (5 micron) column, manufactured by Supelco. Column dimensions were 25 cm by 4.6 mm internal diameter, and the temperature of operation was 30°C. The mobile phase was acetonitrile/water with a flow rate 1.0 mL/min. The detector was a Waters Differential Refractometer Model #R401. The polarity was set to positive, and

the attenuation was fixed at 1×. Peaks were recorded using a Varian Model #9176 strip chart recorder, set at 1.0 cm/min chart speed and 100 millivolt span.

Two ratios of acetonitrile to water were studied (a 60:40 mixture and a 70:30 mixture for the resulting separation on the Supelco column of the five sugars known to be present in soymilk.

Peak identification was based upon a documentation of the retention times of standard solutions of the pure sugars sampled on the HPLC under conditions identical to those used for the test samples. To quantify the results, the peak heights observed, expressed in millimeters, were compared to peak heights recorded for standard solutions of the pure sugars.

Standard solutions of galactose, fructose, sucrose, lactose, raffinose, and stachyose were prepared as aqueous-alcohol solutions (ratio 15 mL alcohol:10 mL water). Concentrations of the standard solutions were 5, 2, 0.5, 0.1, and 0.02 g/L of the respective sugar. These 30 solutions served as standard solutions for peak identification and quantification of the unknown soymilk samples. To generate a standard curve, peak height was recorded for each sugar solution at each level of sugar concentration. These values were tabulated, and least squares analysis was conducted on the regression of peak height versus sugar concentration for each sugar. Since separation was excellent for the conditions of operation, only pure sugar solution standard curves were needed. Consequently, standard curves were constructed for each sugar, i.e., galactose, fructose, sucrose, lactose, raffinose, and stachyose.

Quantification of sugar concentration was based upon peak height measured from the baseline of the chromatograms expressed in millimeters. Accuracy of the peak height method of quantification was considered by application of a sucrose gradient. Three aqueous solutions of sucrose were prepared at 5, 2.5, and 0.5 g/L. A 3 mL sample of each solution was added to 1 mL of soymilk, to give final concentrations of 3.75, 1.88, and 0.38 g/L, respectively. Dilutions were made to keep the peaks within the scale of the strip chart recorder. The soymilk utilized had been prepared from the same batch, thus ensuring that a constant concentration of soymilk sugars would be present in the sucrose plus soymilk mixture. After addition of the sucrose gradient to three 1 mL samples of soymilk, the extraction procedure for sugar recovery, discussed above, was applied to the three sugar-soymilk preparations. Three samples were processed to a clean stream by final treatment with the SEP-PAK cartridges. These cleansed samples were injected one at a time into the HPLC. The intent was to subtract from the sucrose peak arising from the sucrose gradient, the background concentrations of sugars being contributed to the HPLC signal from the soymilk. The resulting concentrations should display the original sucrose gradient.

Reproducibility or precision of the extraction-measurement method was studied by preparing four samples of soymilk obtained from the same batch. The four samples were treated separately using the extraction method of Kwak and

Jeon (1986) to recover the sugars in the milks. A sample of approximately 5 mL was obtained as effluent from the SEP-PAK cartridges for each of the four soymilks; two injections of each soymilk were made into the HPLC. Data were recorded as galactose, sucrose, raffinose, and stachyose peak heights averaged from two injections for each of the four samples. Since the soymilks tested were obtained from the same batch, any observed variation in peak heights in the sugar peaks would be attributed to the extraction procedure.

The effect of pH on quantification and separation of galactose, sucrose, raffinose, and stachyose was studied at the following pH values: the natural pH of soymilk (ca. pH = 6.4); 5.75; 5.70; 5.00; and 4.00. Four soymilk samples were adjusted to the appropriate pH by adding 85 wt.% lactic acid (Fischer Scientific Company A-162). Measurement of pH was made on a Corning pH meter model 7 set to room temperature. Subsequent to pH alteration, the soymilk samples were subjected to the Kwak and Jeon (1986) extraction method and injected into the HPLC. Peak separations and heights were recorded and studied as a function of pH.

The sugar quantification method was checked for dehydration of the milks during the incubation process, which was conducted at 44°C. Four inoculated soymilks of between 27 and 32 g weight were incubated for 6 h at 44°C. The weight of the empty jars and of the jars plus soymilk were monitored by measurement on a OHAUS Dial-O-Gram triple beam balance model #1798 at 0, 4.5, 5.0, 5.5, and 6.0 h. The weight loss attributed to dehydration was expressed in terms of weight percent of the soymilk sample in the jar.

Titratable Acidity

Titration of soymilk was done by addition of 0.1 N NaOH to 15 mL of soymilk or yogurt mix sample. The titration proceeded to a final pH of 7.00 (Buono 1988).

Analysis of Fat Concentration

Fat content of soymilk was measured using the Babcock butter fat test developed for bovine milk (Tamine and Deeth 1980).

Fermentation

Soymilk fermentation and yogurt fermentations were conducted in 4.5 cm diameter by 9.5 cm high cylindrical glass jars obtained from Fischer Scientific. Aseptic conditions were maintained in the jars by use of cheesecloth covered cotton plugs. As shown in Table 1 the method of inoculum preparation and inoculation of the fourth-round of flasks considered above was slightly different in milk and milk mix fermentations (Buono 1988). Two liters of soymilk were

TABLE 1.
MEDIA AND CULTURES OF STUDIES IA THROUGH VI

	Study Code						
	IA	IB	II	III	IV	V	VI
Storage Milk*	NFDM p	NFDM p	SOY p	SOY p	SOY p	NFDM p	SOY p
First-Round Flask	NFDM p	NFDM p	SOY p	SOY p	SOY p	NFDM p	SOY p
Second-Round Flask	NFDM p	NFDM p	SOY p	SOY p	SOY p	NFDM m	SOY m
Third-Round Flask	SOY p	SOY p	SOY p	SOY p	SOY p	SOY m	SOY m
Fourth-Round Flask	SOY m	SOY m	SOY m	SOY m	SOY m	SOY m	SOY m
Storage Time on Soymilk, Hours	0	0	12	168	336	0	168

* NFDM: Nonfat dry milk
* SOY: Soymilk
* p: Pure Culture
* m: Mixed Culture

autoclaved at 121°C for 20 min in a 6 L flask fitted with a cheesecloth covered cotton plug. The inoculum was cultivated in various milks, as discussed by Buono (1988) and summarized in Table 1. Pure cultures of *L. bulgaricus* and *S. thermophilus* (100 mL of each inoculum) were available in two third-round flasks. A 5% volume of total inoculum consisting of a mix from each pure culture flask was added to a sufficient quantity of sterile soymilk in a 6 L Erlenmeyer flask. Pure cultures were mixed in four or five 30 mL inoculum test tubes, which had been sterilized previously, using a Scientific Products S8220 Delux Mixer vortexer so as to introduce a uniformly mixed culture of bacteria into the soymilk. Aseptic transfer was made from the test tubes containing the mixed pure cultures into the 6 L flask containing the sterile soymilk. The entire contents of the 6 L flask were swirled for approximately 1 min. Sterile glass jars were filled aseptically, placed onto a tray and put in an incubator at 44°C.

Sampling was performed every 30 min during soymilk fermentation. One jar was removed at each sample time and served as the sample for that portion of the experiment.

For the HPLC study, extra sample jars were included on the tray. These jars were placed directly from the incubator into a freezer at $-10°C$, where they remained overnight. The next day, the samples were heated for 1 h at room temperature in tap water before undergoing the extraction procedure of Kwak and Jeon (1986). The samples were only partially thawed when the 2-propanol was added.

RESULTS AND DISCUSSION

Measurement of Cell Concentration

Batch growth curves were prepared (Buono 1988) based on measurement of dry weights (DW), viable cell counts (VCC), and direct microscopic counts (DMC) for *L. bulgaricus* and *S. thermophilus,* respectively. Data were plotted as the logarithm of the cell concentration versus time to indicate if the growth of the organisms was exponential in nature. As shown in Fig. 1 and 2, both organisms displayed relatively straight line plots.

In order to measure the concentration of pure culture organisms that were to be used to prepare the mixed culture inocula, a fairly rapid technique was required

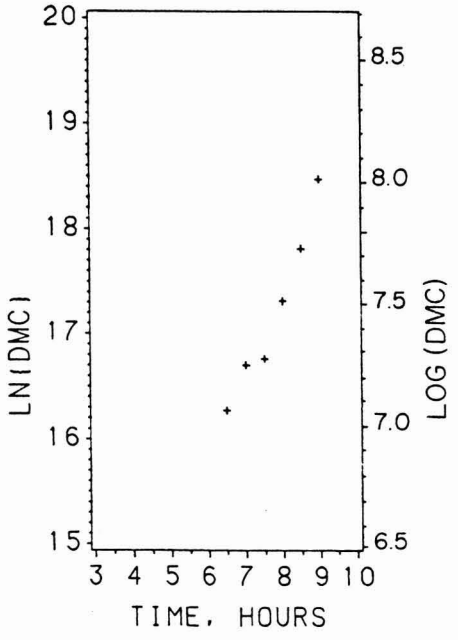

FIG. 1. GROWTH CURVE OF *LACTOBACILLUS BULGARICUS* ON MRS BROTH AT 44°C
DMC represents direct microscopic count expressed in cells/mL.

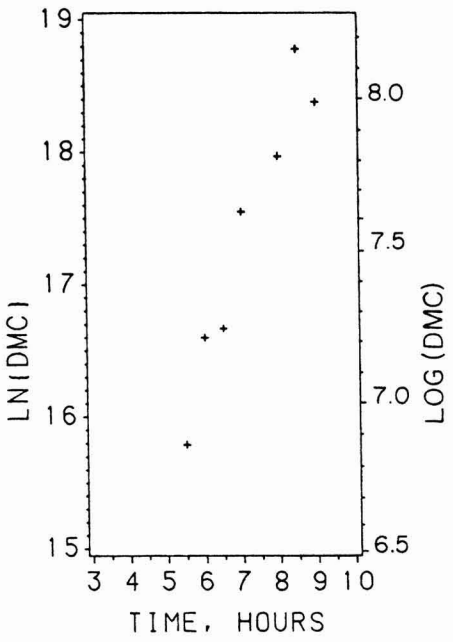

FIG. 2. GROWTH CURVE OF *STREPTOCOCCUS THERMOPHILUS* ON APT BROTH AT 44°C
DMC represents direct microscopic count expressed in cells/mL.

with a small turnaround time. DMC proved most useful, with a turnaround time of approximately 5 min from sampling to measurement. Therefore, the DMC method of measurement was selected for mixed culture inoculum preparation.

Correlations were developed relating ln(DMC) to ln(VCC) and ln(DMC) to ln(DW). For the *L. bulgaricus* and the *S. thermophilus* grown in broths, ln(DMC) was related ln(DW) as follows:

$$\ln(DW) = 0.34 \times \ln(DMC) - 6.99 \text{ for } \textit{Streptococcus}, \; r = 0.91 \quad (6)$$

$$\ln(DW) = 0.31 \times \ln(DMC) - 6.44 \text{ for } \textit{Lactobacillus}, \; r = 0.96 \quad (7)$$

where r is the correlation coefficient.

The key issue to address in the preparation of a yogurt mixed culture inoculum from two pure cultures is: how much of each pure culture should be mixed to yield the final inoculum (Rasic and Kurmann 1984)? The literature indicates that a 1:1 ratio of the two lactic acid bacteria is required for the proper symbiotic growth behavior to result (Matalon and Sandine 1986). However, is this a colony forming unit to colony forming unit ratio, a gram to gram ratio, or a cell to cell

ratio? Experiments were conducted to reveal that the most practical ratio was 1:1 on a gram to gram basis.

In the experiments designed to study the 1:1 ratio, subculturing of inoculum was identical to the broth studies, with the exception that sterile 11% NFDM was used in first- and second-round flasks and fortified soymilk (fortified with nonfat dry milk [NFDM]) was substituted in the third-round flask. Three separate inoculations into three soymilks using the three different 1:1 inoculum schemes were attempted. Growth in the milks inoculated with the 1:1 DMC and 1:1 VCC ratios was unusual for yogurt mixed culture fermentation. In both instances, extremely low maximum counts for the *Streptococcus* (on the order of 10^3 cells/mL) were observed. This was not acceptable, since the symbiotic nature of the yogurt mixed culture requires considerable initial growth of the *S. thermophilus* to stimulate *Lactobacillus* growth. However, in the soymilk inoculated with the gram:gram ratio, growth patterns were as expected for both bacteria. The concentration of *Streptococcus* after approximately 2 h of fermentation was 10^8 cells/mL, and subsequently *Lactobacillus* growth increased and pH decreased. At 4.0 to 6.0 h, the concentration of *Streptococcus* decreased as pH fell. These growth cycles of *Lactobacillus* and *Streptococcus* were quite typical of the microbial activity reported for yogurt (Tamine and Deeth 1980; Oner 1985; Fung *et al.* 1988). Cell counts of the soymilks inoculated with a 1:1 mass ratio displayed an average of 5–12 cocci to 1 rod. As a consequence, DMC was used as the rapid method of bacterial measurement; however, for preparation of the proportions of pure cultures to be mixed to make the inoculum for the soymilk or yogurt mix, dry weight was used in Eq. (2) for variables A and B.

High Performance Liquid Chromatography and Carbohydrate Measurement

Mobile phase composition using the Supelco LC-NH_2 column was seen to significantly affect sugar retention times and separation. Figure 3 displays typical chromatograms obtained after soymilk was treated by the method of Kwak and Jeon (1986) with 2-propanol using two different mobile phases. Part "a" shows the retention times and separation of sucrose, raffinose, and stachyose in a 60:40 acetonitrile:water mobile phase. As seen, the separation of sucrose from stachyose was excellent; however, sucrose and raffinose blended together. Part "b" shows the behavior of an identical soymilk sample in the same Supelco chromatography column with a 70:30 acetonitrile:water mobile phase. An increase in retention times of all sugar peaks was quite evident. This phenomenon was due to increased acetonitrile and decreased water contents in the mobile phase. Also evident was an additional peak, which was identified as a monosaccharide that emerged subsequent to the peak for 2-propanol. All peaks were well separated from each other by retention times of approximately 0.5 to 2.0 min. It was

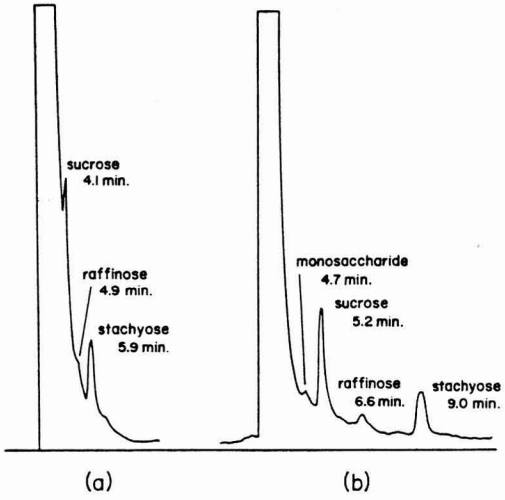

FIG. 3. EFFECT OF ACETONITRILE/WATER MOBILE PHASE CONCENTRATION ON THE SEPARATION OF SOYMILK SUGARS USING HPLC.
a. 60:40 acetonitrile:water
b. 70:30 acetonitrile:water

decided from these and similar studies to conduct all sugar analyses with a 70:30 acetonitrile:water mobile phase concentration.

The sugars of importance were observed to have the following retention times: monosaccharides 4.68 min, sucrose 5.10 min, lactose 6.00 min, raffinose 6.60 min, and stachyose 9.00 min.

Tests for consistency of sugar measurement using HPLC peak height showed that the measured sucrose peak height for unaltered soymilk was 38.3 mm. After addition of the sucrose solution, the corresponding diluted sucrose peak height from the sucrose in the soymilk was 4.8 mm. Sucrose concentrations of 3.75, 1.88, and 0.38 g/L, as measured in soymilk, yielded sucrose peaks of 97.8, 48.8, and 13.8 mm, respectively. Subtraction of the background soymilk sucrose peak, 4.8 mm, gave peak heights of 93.0, 44.0, and 9.0 for 3.75, 1.88, and 0.38 g/L added sucrose, respectively. Least squares analysis applied to a linear regression of peak height against sucrose concentration had a correlation coefficient of 0.9995. The excellent fit of peak height to the sucrose gradient added to soymilk suggested that peak height was suitable for use in quantification of sugars in soymilk.

Examination of residual sugar in the pellet obtained in the extraction process revealed that an 11.7% loss of sucrose had occurred. The average sucrose peak height corresponded to an average concentration of 0.49 g/L. This value can be compared to the average value for sucrose in soymilk of 3.86 g/L.

TABLE 2.
CHECK ON THE EXTRACTION METHOD OF KWAK AND JEON (1986) APPLIED TO SOYMILK

Sample Description: Sucrose concentrations in water-alcohol mixture

Amount of sucrose added (g/l)	Amount of sucrose recovered (g/l)	Percent recovery
10.00	9.86 +/- 0.16	98.6 %
5.00	5.08 +/- 0.12	101.6 %
1.00	0.87 +/- 0.06	87.0 %

Sample Description: Aqueous sucrose solution concentrations before and after extraction by the method of Kwak and Jeon (1986).

Sucrose added to water (g/l)	Sucrose extracted from water (g/l)	Percent recovery
10.00	10.14 +/- 0.13	101.4 %
5.00	5.03 +/- 0.05	100.6 %
1.00	0.91 +/- 0.025	91.0 %

A check was also made on the amount of sugar that was measured in the soymilk sample injected into the HPLC. In the top portion of Table 2, the effect of the denaturing alcohol, 2-propanol, on quantification of the sugar peak is shown to be small. In each instance, except for the lowest concentration of sucrose studied, i.e., 1.0 g/L, the confidence interval included the starting concentration of sucrose. However, for the 1.0 g/L sample, the difference between the expected concentration of 1.0 g/L and the measured value was only 0.07 g/L. Consequently, 2-propanol was deemed an acceptable method of denaturation of soymilk proteins from the standpoint of accurate quantification of sugars in soymilk.

The bottom portion of Table 2 indicates that upon extraction of aqueous solutions of sucrose by the Kwak and Jeon (1986) method, excellent recovery

of the starting sugar concentrations was observed in the effluent streams from the SEP-PAK filters. For a prepared concentration of 10 g/L sucrose and 5 g/L sucrose, recovery by extraction and analysis by HPLC yielded 100%. For the 1.0 g/L prepared concentration, recovery varied from 88.5% to 93.5%. The results indicated that the analytical techniques were accurate for extraction and quantification of carbohydrates in soymilk.

The precision of the analytical techniques was investigated by examining reproducibility using four identical soymilk samples. Table 3 indicates that there were small standard deviations between the four samples for the galactose, sucrose, raffinose, and stachyose HPLC peaks, with highest deviations for the stachyose peak heights. A peak height standard deviation of 2.0, when converted by use of standard concentration (correlation) curves, was equivalent to 0.55 g/L standard deviation associated with the mean stachyose concentration. Since changes in stachyose concentration in the soymilk fermentations studied were greater than the standard deviation, the precision was deemed to be acceptable. Both the sucrose and the galactose standard deviations were small. Sucrose utilization in all data considered was one order of magnitude greater than the standard deviation. The standard deviation for sucrose measurement corresponded to 0.1 g/L sucrose, which can be compared to the sucrose concentrations of 2.00 and 3.00 g/L typically found in soymilk fermentations. The average raffinose peak height measured for four fermented soymilk samples was 8.2 mm larger

TABLE 3.
PRECISION CHECK ON HPLC MEASUREMENT OF CARBOHYDRATES IN FOUR IDENTICAL, FERMENTED SOYMILK SAMPLES

Sample	Peak Height, millimeters*			
	Galactose	Sucrose	Raffinose	Stachyose
1	3.8	4.0	11.0	13.3
2	3.0	4.0	9.8	11.5
3	3.8	4.8	12.5	8.3
4	3.5	4.3	11.8	9.0
Mean	3.5	4.3	11.3	10.5
Std Dev	+/-0.3	+/-0.3	+/-1.0	+/-2.0

* Each value was the average of three measurements.

TABLE 4.
CHECK ON THE EFFECT OF HYDROGEN ION CONCENTRATION ON MEASURED HPLC PEAK HEIGHTS IN UNFERMENTED SOYMILK SAMPLES

Sugar	pH**				
	6.40***	5.75	5.70	5.00	4.00
Galactose	2.8*	2.5	2.5	2.8	2.0
Sucrose	28.0	26.8	27.5	26.0	26.0
Raffinose	3.1	3.0	3.8	4.8	11.3
Stachyose	11.5	11.5	10.8	11.0	11.0

*Peak heights expressed in millimeters.
**pH of soymilk was adjusted by addition of 1.0 M lactic acid.
***6.40 is the normal pH of soymilk.

than the average peak height observed in unfermented soymilk samples. It was determined that the pH of the soymilk had a significant effect on recorded peak height of raffinose. This effect is discussed below.

During the lactic acid fermentation of soymilk, the pH was gradually lowered from approximately 6.4 in the unfermented soymilk to 4.5 in the final yogurt. An experiment was conducted to check the effect of pH on the sugar concentrations measured by HPLC. Protein binding of the sugars can change as the charge on the soymilk proteins varies with pH. This might affect the observed concentrations of the sugars. Table 4 summarizes the effect of pH on observed peak height values recorded for galactose, sucrose, raffinose, and stachyose. Noteworthy was the increase in observed peak height for raffinose from 3.1 mm in natural soymilk with pH of 6.4 to 11.3 mm in soymilk adjusted to pH 4.00. Although there appears to be a significant change in the concentration observed for raffinose, this sugar is known not to be metabolizable by either *Lactobacillus* or *Streptococcus* according to Tamine and Deeth (1980). Thus, the profile of raffinose was not considered in these studies. The average values in Table 3 and Table 4 are identical for the raffinose peaks in fermented soymilk and in pH-adjusted soymilk, respectively. This result indicates that an increase in the HPLC peak height for raffinose was due to the hydrogen ion concentration.

TABLE 5.
WATER EVAPORATION IN FOUR FERMENTING SOYMILK SAMPLES

Sample #	Net Weight (grams)	Weight Loss (grams)	Percent Loss
1	32.20	0.50	1.55
2	27.21	0.13	0.48
3	30.53	0.18	0.59
4	28.87	0.28	0.97

Galactose peak height displayed some decrease with decreasing pH; however, this variation was not of great concern because of the lack of galactose utilization by these lactic acid bacteria, as reported by Tamine and Deeth (1980). Sucrose and stachyose, which were observed to change during lactic fermentation of soymilk, displayed little difference in peak heights for the broad range of pH from 6.40 to 4.00.

During the incubation of soymilk at 44°C, there was some concern as to the effect that dehydration might have had on sugar concentration in the milk. Four fermentations were conducted with soymilk at 44°C in preweighed glass jars. Table 5 lists net weight of each soymilk sample, weight loss from evaporation, and the percent weight loss. The amount of soymilk added to each jar ranged from 27.21 g to 32.2 g. Three of the four soymilks were covered using cotton plugs and aluminum foil, a common practice to ensure asepsis. To determine the effect of the aluminum foil on the total amount of water evaporated from the surface of the soymilks, sample 1 did not have a foil cover. This sample lost more of its mass during fermentation.

Samples 3, 4, and 2, listed in order of decreasing sample weight, were identical treatments except for sample weight. Sample 3 lost 0.59% of its weight to water evaporation, sample 4 lost 0.97% of its weight, and sample 2 lost only 0.48% of its weight. The data in Table 5, therefore, indicate that weight of the soymilk was not an important factor in determining how much water was lost to evaporation.

Sample 1, with no aluminum foil cover over its cotton plug, displayed the greatest amount of evaporation with 1.55% weight loss. This increase in weight loss in the absence of an aluminum foil cover suggested the importance of these

covers for precise and accurate analytical study of concentrations in fermented milk products. The effect of water evaporation on sugar concentration is very small in these experiments.

CONCLUSIONS

(1) Direct microscopic counting (DMC) of lactic acid bacteria was made in milk systems with a turnaround time of 10 min. This method of bacterial enumeration can be used successfully to determine cell concentrations in pure culture inoculum flasks.

(2) Correlation between direct microscopic count (DMC) and biomass dry weight (DW) was determined for *Lactobacillus bulgaricus* and for *Streptococcus thermophilus;* see Eq. (6) and (7).

(3) A 1 g to 1 g ratio of pure cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* was used to prepare the mixed culture inoculum. This mass ratio yielded between 5–10 cocci to every one rod cell in the mixed culture inoculum. This ratio was superior to a 1:1 cell number ratio.

(4) Kwak and Jeon's (1986) method for sugar extraction from bovine milk was successfully applied to soymilk. In addition, checks for recovery of sugars from pellet and supernatant, concentration effect of dehydration, pH, and precision of the measurement between samples all proved positive, varifying the applicability of this technique to soymilk fermentations.

(5) Less than 1% of the mass evaporated during fermentation for samples covered with cotton plugs and aluminum foil.

NOMENCLATURE

A = microscopic count or dry weight or colony forming unit count of *Streptococcus,* number/L or g/L or CFU/L
B = cell count or dry weight or colony forming unit of *Lactobacillus,* number/L or g/L or CFU/L
C = cell concentration measured by direct microscopic counting, cells/mL
D = diameter of microscope field of view, cm
DW = biomass dry weight, g/L
DMC = direct microscopic count of cocci and rods, number of cocci or rods/mL
V = volume of soymilk to be inoculated, mL
VCC = viable cell count, colony forming units/mL
X = volume of *Streptococcus* inoculum, mL
Y = volume of *Lactobacillus* inoculum, mL

ACKNOWLEDGMENT

The long hours of support in sample preparation and the consultation with food science and other graduate students are greatly appreciated. Further, we thank the U.S.D.A. for providing stipend support to M. A. Buono throughout the course of this study (National Needs U.S.D.A. Graduate Fellowship).

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EFFECT OF CORN GERM PROTEIN ON THE QUALITY CHARACTERISTICS OF BEEF PATTIES HEATED BY MICROWAVE[1]

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Accepted for Publication July 12, 1989

ABSTRACT

Beef patties were extended at levels of 10, 20, and 30% of the uncooked weight with a slurry of defatted corn germ protein (CGP) and heated by microwave. Extended patties had lower heating losses and higher yields than control patties. Incorporation of CGP in beef patties lowered protein and fat contents, increased moisture contents, increased water and fat retention, decreased cohesiveness of beef patties, but did not affect hardness. Addition of CGP affected sensory characteristics: meaty aroma and flavor decreased with an increase from 10 to 30% CGP. Corn germ protein is recommended as an extender in coarsely ground meats.

INTRODUCTION

There have been numerous efforts to increase the use of nonmeat proteins in meat products because of their potential to lower production costs (Cross *et al.* 1975; Smith *et al.* 1973; Wills and Kabirullah 1981). Use of soy-based products, including soy flours, concentrates, and isolates, is well-known (Keeton *et al.* 1984; Parks and Carpenter 1987). Other oilseed proteins, including peanut and cottonseed (Terrel *et al.* 1981), sunflower (Wills and Kabirullah 1981) and navy bean (Patel *et al.* 1980) also have been processed into food-grade ingredients and studied as additives in meat products. However, there are limited data related to the use of high protein food ingredients derived from cereal grains.

[1]Contribution No. 89-350-J from the Kansas Agricultural Experiment Station, Manhattan, Kansas 66506

Journal of Food Processing and Preservation **13** (1990) 155–168.

The major food products of the corn dry milling industry come from the endosperm portion of the corn kernel, resulting in 65–70% of the industry's output. In this processing technique, the germ portion of the kernel is an important intermediate product, since it undergoes further processing for oil extraction. The corn germ meal, with a white to cream color, that remains following oil extraction can be processed into a defatted corn germ product. Because of its relatively high protein content (24–26%), the resulting product is referred to as corn germ protein (CGP).

The degree to which the fat is extracted from corn germ meal is important not only for the yield of corn oil but also for the storage stability of the resulting CGP. Lipids in CGP will either enzymically or autooxidize into off-flavor compounds during storage, which will reduce the flavor and nutritional quality of the product (Christianson *et al.* 1984). Conventional hexane extraction leaves residual lipids in CGP (Phillips and Sternberg 1979; Christianson *et al.* 1984). Supercritical-CO_2 extraction is more efficient for the removal of triglycerides and bitter constituents (bound lipids), as well as for the inactivation of peroxidase enzymes, thereby maintaining the flavor and storage stability of CGP (Christianson *et al.* 1982; Christianson *et al.* 1984). However, a modified process for hexane-extraction of oil from corn germ meal has been developed. This method significantly improved sensory characteristics of flavor and color of CGP. A modified fat extraction technique was effective in producing CGP with a low fat content (0.2% and less), and high storage stability (Zayas and Lin 1988). This CGP was tested as an extender in comminuted meats (Lin and Zayas 1987a; Zayas and Lin 1988).

A potential use of CGP is in institutions like hospitals and schools that must provide nutritious meals under food budget restraints. The trend toward more away-from-home eating also is likely to increase the potential market for extenders. The success of nonmeat proteins in ground beef products will depend upon whether the finished product resembles the traditional beef patty. Corn germ is known for its high nutritional value; however, little information is available as to the actual quality effects that result from extending ground beef with CGP.

The objective of this study was to assess the effects of extending ground beef patties with CGP, to determine the effect of CGP on heating losses and quality characteristics (proximate analysis, textural and sensory properties) of beef patties heated by microwave.

MATERIALS AND METHODS

Sample Preparation

Meat trimmings were purchased from a local distributor (Flint Hills Foods, Alma, KS). A flow-diagram for preparation of samples is illustrated in Fig. 1.

Fat (50/50) and lean (90/10) sources were ground separately through a 1 in (2.54 cm) break plate. A Pearson square calculation was used to determine the amounts of fat and lean portions needed to formulate the targeted fat content of 20%. A Hobart fat tester (Hobart Corporation, Troy, OH) was used to ensure that the ground product met the targeted fat level.

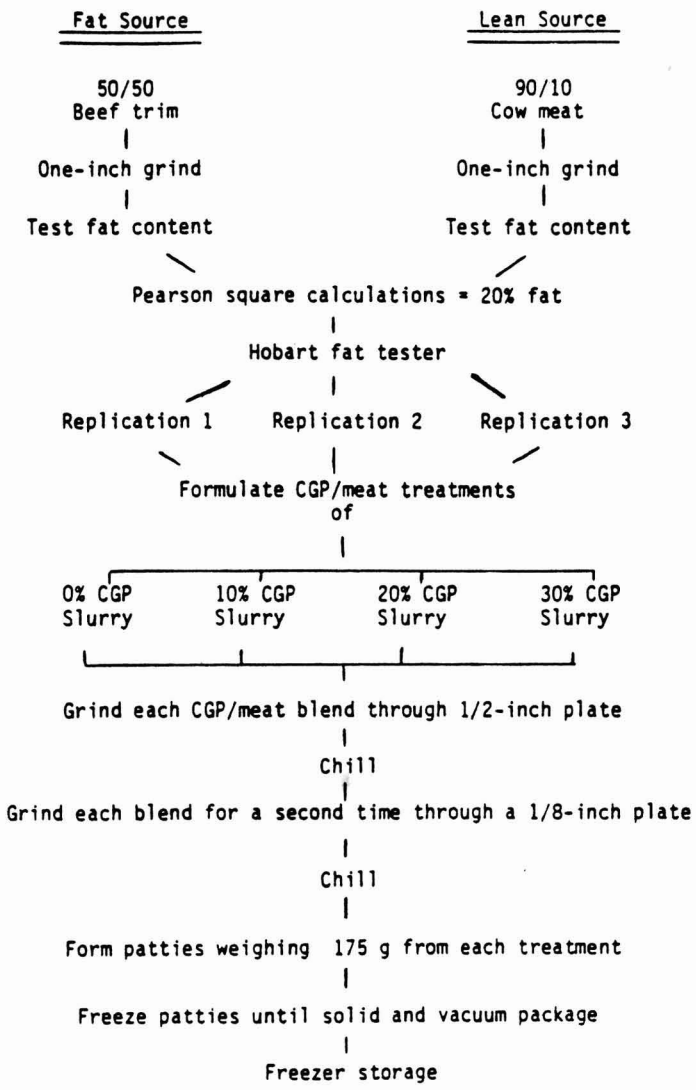

FIG. 1. FLOW-DIAGRAM FOR PREPARATION OF EXPERIMENTAL AND CONTROL SAMPLES

The ground meat with controlled fat content obtained from three lots of meat trimmings was blended and divided into three separate batches corresponding to the three replications of the study, vacuum packaged, and held in a walk-in cooler at 4°C until treatments were prepared.

Treatments

Beef patties were extended with rehydrated corn germ protein (CGP) at levels of 10, 20, and 30% of the raw weight of the total mix, resulting in actual CGP concentrations of 2.5, 5.0, and 7.5% of the total mix (Table 1). Control samples were prepared without CGP. Formulations of patties are presented in Table 1. To prepare the CGP slurries, dry CGP was hydrated with distilled water at a ratio of 1:3 in glass beakers. These were covered with plastic wrap and held at room temperature for 90 min. Hydrated CGP was mixed with the meat and ground through a $\frac{1}{2}$ in (1.27 cm) plate, to more thoroughly incorporate the CGP throughout the product.

The ground beef/CGP mixture was chilled in a freezer for 10 min to lower the temperature of the mix before grinding for a second time. The chilled mix was passed through an $\frac{1}{8}$ in (0.32 cm) plate and again chilled for 10 min. The chilled mixture was weighed into 175 g portions and formed into patties using a hand press (Tupperware, Orlando, FL), sprayed with a nonstick spray for easy release. Formed patties were placed between squares of waxed paper, layered on metal trays, and frozen solid. Frozen patties were vacuum packaged in groups of three and stored at −23°C. Patties were thawed overnight in a 4°C refrigerator before heat treatment.

TABLE 1.
FORMULATIONS OF BEEF PATTIES, WITH AND WITHOUT CORN GERM PROTEIN

Ingredients	CGP Slurry			
	0%	10%	20%	30%
Ground Beef, g	2562	2492	2212	1932
Salt, g	28	28	28	28
Water, g	210	210	420	630
CGP[1], g	--	70	140	210
	2800	2800	2800	2800

[1]CGP = Corn germ protein.

Heating

A carousel microwave oven (Sharp Electronics Corp., Paramus, NJ, Model R-8200) was used as the heating source in this cookery method. In order to make a browned crust on the patty surfaces, a browning griddle (Corning Glass Works, Corning, NY, MW-11) was used following manufacturer's directions. Patties were cooked for 3 min, turned over, and heated at 1 min intervals until an internal endpoint temperature of 77°C was obtained. Weights of patties before and after cooking were obtained for purposes of calculating heating losses and cooked yields. Measurements of total cooking losses were separated as drip losses and volatile losses.

Proximate Analysis

Moisture content of raw and cooked samples was determined by drying them in an oven overnight at 105°C (AOAC 1984, Method 24.002). Fat content was determined using the Foss-Let fat analyzer (A/S N. Foss Electric, Denmark) with a 22.5 g sample of ground beef patty being reacted for 2.5 min with 120 mL tetrachloroethylene and 50 g plaster of paris in the reaction chamber. Extracted filtrate was analyzed for a direct reading of fat content (AOAC 1984, Method 24.006).

The methods of Anderson and Lind (1975) were employed to determine differences in the retention of fat and water in beef patties. Percentages of water and fat retention were calculated:

$$\% \text{ water (fat) retention} = \frac{\% \text{ cooked yield} \times \% \text{ cooked moisture (fat)}}{\% \text{ uncooked moisture (fat)}}$$

Percent nitrogen was analyzed by the Buchi method (modified Kjeldahl) with a conversion factor of 6.25 being utilized to convert the data to percent protein (AOAC 1984, Method 24.028).

Sensory Evaluation

A six-member, professional panel was trained in two sessions to become familiar with the product's characteristics of aroma, flavor and texture. Aromatic notes evaluated included meaty aroma, off-aroma, and off-aroma acceptability. Flavor notes included meaty flavor, off-flavor and off-flavor acceptability. Textural attributes evaluated were juiciness, mushiness, and grind. Perceived grind was determined visually. All treatments utilized the same grinding technique. An evaluation of off-color was made as well. All parameters were scored on a 6-in. (15 cm), unstructured, intensity line scale with anchors at each endpoint

and a centerline. The results were converted into numbers by measuring the distance of the line scores from the left end of the line scale.

Textural Analysis

Shear values and resistance to compression were measured on cooked patties at ambient temperature using a Universal Instron Testing Machine (Model 1122). Analyses of shear force and compression were carried out on the same patty. The upper crust of each patty was removed with use of a miterbox to make patties of uniform thickness, 1.81 cm. Hardness was measured by the height of the resulting curve and was expressed as kg force. Compression data were obtained using the flat-headed plunger attachment (diameter, $1\frac{3}{8}$ in.) to the Instron. Cores of 1.81 cm diameter were removed from each patty. The force necessary to compress the samples by 54% of original thickness was measured twice on each core. As a measure of the binding ability of CGP in beef patties, cohesiveness was determined by a compression test. Cohesiveness was measured as the ratio of the area under the second compression force curve (A_2) to the area under the first curve (A_1), as described by Bourne (1978).

Color Measurement

Cooked patties, which had been finely ground with a food processor, were used for the Hunterlab color measurement (Hunterlab D54 Spectrophotometer, Hunter Associates Laboratory, Fairfax, VA). L, a, and b values using illuminant A and C light sources were obtained. These data then were used to calculate the indices of saturation as follows (Little 1975):

$$\text{Saturation Index: } S = (a^2 + b^2)^{1/2}$$

Statistical Design

A randomized block design was used, and data were analyzed for differences due to level of CGP extension, using analysis of variance (Steel and Torrie 1980). For each source of variation for which the F-value was significant, the least significant difference at the 5% level of probability was calculated. Correlations between selected measurements were determined.

RESULTS AND DISCUSSION

Heating Losses

Volatile losses were measured and they consisted primarily of evaporated water that was released from the meat tissue as proteins became denatured and

TABLE 2.
HEATING LOSSES OF MICROWAVED BEEF PATTIES WITH AND WITHOUT ADDED CORN GERM PROTEIN (CGP)

Added CGP Slurry[d] (%)	Heating Losses (%)		
	Volatile	Drip	Total
0	26.33[a]	10.11[a]	36.32[a]
10	25.87[a]	8.15[a,b]	34.02[a]
20	23.25[a]	6.96[b,c]	30.21[b]
30	23.26[a]	5.68[c]	28.94[b]

[a,b,c]Means in the same column with the same superscript letters are not different ($P<0.05$).

[d]CGP slurry = corn germ protein slurry; CGP hydrated with distilled water in ratio of 1:3.

coagulated. Volatile losses also included aromatic compounds, primary and secondary products of heat decomposed fat, and fat droplets that have spattered out of the pan. Drip losses were measured and they included primarily fat melted out of the meat tissue during heating, water and nonvolatile water soluble materials, such as salts and sarcoplasmic proteins (Paul 1972). Heating losses of microwave treated beef patties are shown in Table 2. Adding corn germ protein (CGP) slurry at 20 and 30% levels decreased ($P < 0.05$) total cooking losses in microwave heated beef patties (Table 2). The overall trend was for reduction in heating losses as the level of added CGP increased. Mean values for total heating losses were greater ($P < 0.05$) for the control and 10% CGP extended than for samples containing 20 and 30% CGP. There was no difference in volatile losses between the all-meat control and CGP extended patties. However, drip losses were reduced in microwave heated patties with 20 and 30% CGP added.

Proximate Analysis

Fat and water contents were determined in both raw and cooked patties containing CGP (Table 3). Experimental data showed that the protein content of microwave heated patties decreased ($P < 0.05$) with the addition of CGP at all three levels (Table 3). In raw patties, there was a decrease in protein content at the 20 and 30% extension levels. This may seem surprising since CGP contains 24–26% protein and the meat it is replacing only contains 14% protein. However, this is explained by a balance change between basic components and a significant

TABLE 3.
PROXIMATE COMPOSITION, YIELD, FAT AND WATER RETENTION OF MICROWAVE HEATED BEEF PATTIES WITH AND WITHOUT ADDED CORN GERM PROTEIN (CGP)

Added CGP Slurry[e] (%)	Protein (%)		Fat (%)		Water (%)		Yield	Retention (%)	
	Raw	Cooked	Raw	Cooked	Raw	Cooked	(%)	Fat	Water
0	14.85[a]	24.11[a]	19.32[a]	17.78[a]	62.71[a,b]	55.70[a]	63.68[a]	57.54[a]	55.47[a]
10	14.88[a]	22.28[b]	18.64[a,b]	16.10[b]	61.94[a]	56.44[a,b]	65.98[a]	58.64[a,b]	60.56[b]
20	14.19[b]	19.89[c]	17.08[b]	15.62[b]	62.17[a,b]	56.93[b]	69.79[b]	63.93[a,b]	63.97[c]
30	13.54[c]	18.23[d]	14.38[c]	13.73[c]	63.92[b]	57.29[b]	71.06[b]	68.90[b]	64.40[c]

[a,b,c,d]Means in the same column with the same superscript letters are not different ($P<0.05$).

[e]CGP slurry = corn germ protein slurry; CGP hydrated with distilled water in ratio of 1:3.

increase of carbohydrates in the formulation. An additional factor is a dilution effect on the protein, when the CGP additive is hydrated with an amount of water that is three times its weight. Therefore, the reduction in protein content resulted from the higher ($P < 0.05$) retention of fat and water in patties extended with CGP (Table 3).

Nutritional guidelines recommend a reduction in the caloric intake from fat. Results indicated that manufacture of low-fat beef patties is possible with the utilization of plant proteins such as CGP. Addition of a low-cost, defatted CGP not only will lower production costs by replacing meat proteins and increasing yields, but will improve the nutritional quality of the product by lowering its fat content. Extension with defatted corn germ protein decreased ($P < 0.05$) fat content in raw beef patties. Following microwave heat treatment, beef patties showed significant differences for fat content. Fat content in microwave heated beef patties containing added CGP was significantly lower ($P < 0.05$) than that of the control patties for all three extension levels (Table 3). At the same time, beef patties with 10 and 20% CGP contained more fat than those with 30% CGP. This was due to the dilution effect of adding defatted CGP to ground beef during formulation.

A desirable nutritional characteristic of CGP is its low fat content. However, this positive quality would be partially negated if beef patties extended with CGP retain a greater percentage of fat in cooking than all-meat patties. Measurements of fat and water content and their retention following microwave heat treatment are presented in Table 3. Compared with the all-meat control, fat retention increased at the 30% of CGP extension level (Table 3) and accounted for some of the decrease in drip loss. However, there was no significant difference ($P < 0.05$) in fat retention between the all-meat control and beef patties extended with 10 and 20% CGP. Corn germ protein increased the degree of fat globule stabilization by forming a protein film on the surface of fat droplets, which prevented coalescence during heat treatment (Lin and Zayas 1987b).

Microwave heated beef patties with 20 and 30% added CGP had higher ($P < 0.05$) moisture content than the all-meat control (Table 3). There was no difference in moisture content between the control and patties with 10% CGP. Therefore, it can be concluded that water retention also played a significant role in reducing drip losses during heating.

An important property of a protein intended as a meat additive is the ability to increase water-holding capacity. Corn germ protein has been referred to as a protein source of high water-holding capacity (Lucisano *et al.* 1984), similar to that of soy concentrate (Phillips and Sternberg 1979). Addition of CGP slurries at three levels significantly increased ($P < 0.05$) the water retention of microwave heated beef patties (Table 3). Anderson and Lind (1975) found that the percent of water retention was greater for cooked beef patties containing textured soy

protein. Retention of fat and moisture was directly related to the percent of textured vegetable protein present in the patties. Our results (Table 3) showed that CGP has the ability to increase both fat and water retention, with a higher value for fat retention (68.90%) than for water retention (64.40%) at the 30% CGP extension level.

Data were collected to determine the effects of CGP extension on yield of microwave heated beef patties. Addition of 20 and 30% CGP was found to increase cooking yields in microwave heated beef patties. There was no difference in mean values for percent yield between the control patties and the 10% CGP treatment level. The yields with 20 and 30% CGP were 6.11% and 7.38% higher than that of the control, respectively. Although not significantly different, the trend was for increasing percent yield as the level of CGP extension increased, because of higher water and fat retention.

Textural Characteristics of Beef Patties

Hardness and cohesiveness were determined on microwave cooked samples as kg force for compression. Instrumental measurements for hardness of cooked beef patties showed slightly lower scores as the amount of added CGP slurry in the mixture increased (Table 4), but differences were not significant. Cross *et al.* (1975) reported that the factors responsible for toughness in ground beef are related to the myofibrillar and stromal proteins. Addition of CGP would have a

TABLE 4.
TEXTURAL PROPERTIES OF MICROWAVED BEEF PATTIES WITH AND WITHOUT CORN GERM PROTEIN (CGP)

Added CGP Slurry[c] (%)	Compression	
	Hardness (Kg)	Cohesiveness (Peak II: Peak I)
0	2.42[a]	.76[a]
10	2.19[a]	.71[a,b]
20	2.12[a]	.63[b]
30	1.65[a]	.60[b]

[a,b,c]Means in the same column with the same superscript letters are not different ($P<0.05$).

[d]CGP slurry = corn germ protein slurry; CGP hydrated with distilled water in ratio 1:3.

TABLE 5.
TRISTIMULUS COLOR VALUES OF MICROWAVED BEEF PATTIES WITH AND WITHOUT CORN GERM PROTEIN (CGP)

Added CGP Slurry[c] (%)	L	a	b	Saturation Index
		Illuminate A		
0	46.34[a]	7.18[a]	4.25[a]	8.34[a]
10	44.39[a]	6.10[a]	4.02[a]	7.30[a]
20	45.78[a]	5.82[a]	4.03[a]	7.08[a]
30	45.64[a]	5.73[a]	4.38[a]	7.21[a]
		Illuminant C		
0	45.34[a]	3.01[a]	7.52[a]	8.11[a]
10	43.54[a]	2.38[a,b]	7.18[a]	7.57[a]
20	44.90[a]	2.24[a,b]	7.17[a]	7.52[a]
30	44.78[a]	1.99[b]	7.81[a]	8.07[a]

[a,b]Means in the same column with the same superscript letters are not different ($P<0.05$).

[c]CGP slurry = corn germ protein slurry; CGP hydrated with distilled water in ratio of 1:3.

diluting effect on these proteins and thus might increase tenderness. Beef patties with 20 and 30% CGP had lower cohesiveness or binding ability than all-meat control patties (Table 4). The development of methods that optimize the extraction of meat proteins could presumably improve the binding capacity of CGP-extended ground meat products.

Color Determination

The objective of the color evaluation was to detect tendencies for CGP addition to change the apparent color of the microwave heated beef patties. Results of color measuring are presented for microwave heated beef patties in Table 5 (illuminate A and C). The results did not reveal any differences ($P < 0.05$) in the amount of color or its intensity within the treatments evaluated under the

TABLE 6.
AROMA CHARACTERISTICS OF MICROWAVED BEEF PATTIES WITH AND WITHOUT CORN GERM PROTEIN (CGP)

Added CGP Slurry[d] (%)	Meaty Aroma[e]	Off-Aroma[e]	Off-Aroma Acceptability[f]
0	4.49[a]	0.97[a]	4.73[a]
10	2.86[b]	2.91[b]	3.70[b]
20	1.77[c]	3.94[c]	3.31[b,c]
30	0.94[c]	4.66[c]	2.86[c]

[a,b,c]Means in the same column with the same superscript letters are not different ($P<0.05$).

[d]CGP slurry = corn germ protein slurry; CGP hydrated with distilled water in ratio of 1:3.

[e]Based on six-inch sensory line-scale anchored on opposing ends—"none" (0) and "intense" (6)—with midpoint marked. Measured to 1/16 of an inch.

[f]Based on six-inch sensory line-scale anchored on opposing ends—"dislike" (0) and "like" (6)—with midpoint marked. Measure to 1/16 of an inch.

light source of illuminate A (Table 5). Extended beef patties were not different ($P < 0.05$) in lightness (L values) and red (+a value) color than the control. No differences were noted in the amount of yellowness (+b value) among the patties with either light source. This suggests that CGP, a white-yellow compound had no effect on the color of beef patties. Data for illuminate C showed a decrease ($P < 0.05$) in red color at the 30% CGP extension level (Table 5).

Sensory Evaluation

Sensory evaluation data are presented in Tables 6 and 7. Panelists found the aroma of all-meat control patties to be more acceptable than that of all three experimental samples. Meaty aroma and off-aroma acceptability decreased ($P < 0.05$) with increasing levels of CGP and, conversely, off-aroma of the extended beef patties increased with increasing levels of CGP (Table 6).

The attributes of meaty flavor and off-flavor acceptability decreased ($P < 0.05$) with increasing levels of added CGP, whereas the attributes of off-flavor and

TABLE 7.
SENSORY PROPERTIES OF MICROWAVED BEEF PATTIES
WITH AND WITHOUT CORN GERM PROTEIN (CGP)

Added CGP Slurry[e] (%)	Meaty Flavor[f]	Off-Flavor[f]	Off-Flavor Acceptability[g]	Juiciness[f]	Tenderness[f]	Mushiness[f]	Grind[h]	Off-Color[f]
0	4.76[a]	0.73[a]	4.79[a]	3.41[a]	2.83[a]	1.02[a]	3.76[a]	0.64[a]
10	2.86[b]	2.86[b]	3.73[b]	2.91[a]	2.92[a]	2.27[b]	2.95[a,b]	1.42[b]
10	1.51[c]	4.22[c]	3.07[c]	2.83[a]	3.68[a,b]	2.86[c]	2.36[a,b]	1.14[b]
30	0.85[d]	5.08[d]	2.48[d]	3.54[a]	4.14[b]	3.82[d]	1.54[b]	1.10[b]

[a,b,c,d]Means in the same column with the same superscript letters are not different ($P<0.05$).

[e]CGP slurry = corn germ protein slurry; CGP hydrated with distilled water in ratio of 1:3.

[f]Based on six-inch sensory line-scale anchored on opposing ends—"none" (0) and "intense" (6)—with midpoint marked. Measured to 1/16 of an inch.

[g]Based on six-inch sensory line-scale anchored on opposing ends—"dislike" (0) and "like" (6)—with midpoint marked. Measured to 1/16 of an inch.

[h]Based on six-inch sensory line-scale anchored on opposing ends—"fine" (0) and "coarse" (6)—with midpoint marked. Measured to 1/16 of an inch.

mushiness increased (Table 7). The perception of off-flavor increased ($P < 0.05$) and the evaluation for meaty flavor decreased as the amount of added CGP increased. This indicated that the CGP additive affected the flavor of beef patties. Sensory panel results proved that panelists could detect an off-flavor at the 10% level of added CGP slurry (only 2.5% CGP on a dry weight basis), with the acceptability of the off-flavor decreasing as the levels of extension increased.

Juiciness did not differ significantly for CGP-extended and control patties. Even though CGP-extended beef blends contained more moisture than control beef patties, adding CGP did not affect juiciness.

Addition of CGP had an increasing effect on the tenderness of beef patties, which was greatest at the 30% CGP extension level. Although all treatments were prepared in the same manner, panelists perceived the grind (particle size) of beef patties containing CGP to be finer than that typical of ground beef (Table 7). Data for sensory evaluation showed that panelists were capable of detecting a slight off-color in the patties extended with CGP (Table 7), with ratings remaining at the lower end of the sensory line-scale.

An important result is that the panelists' evaluations of off-aroma acceptability and off-flavor acceptability of microwave-heated beef patties with up to 20% CGP never crossed the midpoint of the line-scale into the "dislike" region. Therefore, no objectionable flavor and aroma were found in these beef patties. Patties with 30% CGP extension (7.5% on a dry basis) did fall within the "dislike" region of the sensory scale, having an average value of 2.48 for off-flavor acceptability and 2.86 for off-aroma acceptability.

CONCLUSIONS

Utilization of defatted corn germ protein (CGP) increased the yield of microwave-heated beef patties as the result of increased water and fat retention and decreased heating losses. Protein and fat contents of both raw and microwave heated CGP-extended beef patties decreased with increasing levels of CGP. This was due to a dilution effect from the replacement of meat protein with a slurry of hydrated CGP. Incorporation of CGP did not affect hardness but decreased cohesiveness of microwave-heated beef patties. Addition of 30% CGP increased patty tenderness but had no effect on the juiciness. There was no difference in the amount of color and its intensity within the treatments evaluated under illuminate A, but a decrease in red color was noted under illuminate C. Addition of CGP in the formulations of beef patties affected sensory characteristics, especially aroma and flavor. Meaty aroma and flavor decreased with an increase in added CGP from 10 to 30%. Microwave heated beef patties extended with CGP slurry had an off-aroma and off-flavor, which increased in intensity with increasing levels of extension. However, no objectionable aroma and flavor were

found in beef patties with up to 20% CGP. Based on these experimental data, CGP can be recommended as an extender in microwave-heated beef patties.

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

Description of experimental work or explanation of symbols should go below the table proper.

Figures should be listed in order in the text using Arabic numbers. Figure legends should be typed on a separate page. Figures and tables should be intelligible without reference to the text. Authors should indicate where the tables and figures should be placed in the text. Photographs must be supplied as glossy black and white prints. Line diagrams should be drawn with black waterproof ink on white paper or board. The lettering should be of such a size that it is easily legible after reduction. Each diagram and photograph should be clearly labeled on the reverse side with the name(s) or author(s), and title of paper. When not obvious, each photograph and diagram should be labeled on the back to show the top of the photograph or diagram.

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Short notes will be published where the information is deemed sufficiently important to warrant rapid publication. The format for short papers may be similar to that for regular papers but more concisely written. Short notes may be of a less general nature and written principally for specialists in the particular area with which the manuscript is dealing. Manuscripts which do not meet the requirement of importance and necessity for rapid publication will, after notification of the author(s), be treated as regular papers. Regular papers may be very short.

Standard nomenclature as used in the biochemical literature should be followed. Avoid laboratory jargon. If abbreviations or trade names are used, define the material or compound the first time that it is mentioned.

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JOURNAL OF FOOD PROCESSING AND PRESERVATION VOL. 14, NO. 2

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