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EDITORIAL

Volume 13 of the *Journal of Food Processing and Preservation* is now history and I can report that we have had a busy and successful year. Although we did not have many papers in our two unique sections entitled "Computer Codes and Their Applications" and "Databank", we have had excellent research contributions. As in the past two years, I would like to encourage our readership to submit papers in both the Computer Codes and Databank sections.

This year, I would especially like to thank Ms. Kathy O'Neil, Food & Nutrition Press, for her excellent effort on behalf of the *Journal of Food Processing & Preservation*. The entire operation is a team effort which includes authors, reviewers (see list below) and publisher. I would like to extend a thank you to all of those who participated in maintaining the quality reputation that the journal enjoys. I would like to thank the Editorial Board for their effort on behalf of the *Journal of Food Processing & Preservation*, especially Drs. Karel, Satterlee, Toledo and Wrolstad who have completed three year terms.

I appreciate the support which we have received for the journal. As we complete our first year of six issues per year, we can look forward to serving our profession with the continued high-quality publication.

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DARYL LUND
Editor

THE 1990 GEDDES MEMORIAL LECTURESHIP

The Northwest Section of the American Association of Cereal Chemists is seeking nomination from all AACC Sections or AACC members for the 1990 William F. Geddes Memorial Lectureship. The lecturer is invited to address the Section at a regular meeting to speak on advances in plant breeding, formulations, processing, nutrition value or other aspects of science and technology of the cereal grain or other plant crops or the products of which plant crops are significant constituents. The individual receives a $500.00 honorarium, an inscribed plaque, and necessary travel expenses. The lectureship honors the William F. Geddes achievement and memory, a former member of the section and professor of the University of Minnesota. Nomination forms are available from Dr. Mary K. Schmidl, Sandoz Nutrition, 5320 W 23rd Street, Minneapolis, Minnesota 55416. Deadline is December 31, 1989.

CONTENTS

SORBIC ACID STABILITY DURING PROCESSING AND STORAGE OF AN INTERMEDIATE MOISTURE CHEESE ANALOG[1]

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ABSTRACT

The chemical stability of sorbic acid during processing and storage of intermediate moisture foods was determined using a soybean based cheese analog. Samples prepared with 0.57 to 4.24% homogeneously distributed K-sorbate showed losses of less than 25% after 40 days storage at 35°C, 88% RH air and in the dark. Heating (2 h in an 85°C water bath) resulted in no significant sorbic acid losses. Samples with surface applications of 1 mg sorbic acid/cm^2 showed no detectable losses after 10 days storage under the same conditions. Furthermore, the stability of the preservative was not affected by growth of Staphylococcus aureus *S-6.*

INTRODUCTION

Sorbic acid is often used in intermediate moisture food (IMF) formulations as a fungistatic agent. It has also been found to be effective against *Staphylococcus aureus* (Pierson *et al.* 1979; To and Robach 1980; Robach and Stateler 1980; Robach and Sofos 1982; Elliot *et al.* 1982; Parada *et al.* 1982). *S. aureus* presence in IMF is of significance because of its pathogenic character and its ability to survive low water activity (a$_w$) environments (Scott 1953; Pawsey and Davies 1976). Although the stability of sorbic acid has been studied previously, (e.g., Heintze 1976; Bolin *et al.* 1980; Saxby *et al.* 1982) we found no reports on the effect of processing conditions such as heating, moisture removal by warm air; the stability difference between surface and bulk applications; nor the effect

of storage time and microbial growth when related to IMF products. This study focused research on these questions using an IM cheese analog prepared as described elsewhere (Motoki *et al*. 1982; Torres *et al*. 1985).

MATERIALS AND METHODS

Initial potassium sorbate concentrations were selected to cover the 0.5 to 4.2% (w/w, wet basis) range. Higher concentrations than the normal upper limit usage level (0.3%) (Anon. 1978a) were included to simulate the high local concentrations existing in surface applications. Samples with sorbic acid applied as a surface spray were included to determine if surface conditions accelerated sorbic acid degradation. To maintain a higher surface concentration during storage, samples were precoated with a zein film prepared as reported by Torres *et al*. (1985). Zein films reduce sorbic acid diffusion from surface into food bulk (Torres 1987). Samples were also inoculated with *S. aureus* S-6 (Torres and Karel 1985) to determine whether its growth affected sorbic acid stability.

Table 1 shows the components of the IM cheese analog prepared as described by Motoki *et al*. (1982) and Torres *et al*. (1985). The initial moisture content of this analog was 100 cm^3/100 g solids and was reduced to $a_w = 0.88$ by blowing warm air into the food cutter where the ingredients were mixed. The a_w was measured using an electric hygrometer (SINA Equihygroscope, Beckman Industrial, Cedar Grove, NJ). Samples were then stuffed into cellulose casing

TABLE. 1.
INTERMEDIATE MOISTURE CHEESE ANALOG

Ingredient	% w/w
isolated soy protein	26.5
Na-caseinate	6.0
Ca-caseinate	2.0
hydrogenated vegetable oil	34.6
decaglycerol mono oleate	0.4
salt	4.9
glycerol	6.0
sorbitol	19.6
K-sorbate	variable

(Type 30F0, Union Carbide, Tarrytown, NY) with a hand-press. Thereafter, casings were placed into seamless polyvinylidene chloride casing tubes (diameter 40mm, Kreha Chemical Co., Tokyo, Japan) and then heated for 2 h in a water bath kept at about 85°C. After heating, a_w was measured again with the electric hygrometer. Sample pH was determined with a surface electrode probe (combination electrode 39507, Beckman Instruments, Inc., Cedar Grove, NJ). Casings were then carefully removed under a laminar air flow hood and the cylinders thus obtained were cut into disks. Each disk was placed on a sterile dissecting needle and stored in a constant 88% RH chamber (over saturated $BaCl_2$). The chamber itself was placed in a constant temperature room at 35°C. Air in the chamber was kept in circulation by the use of a fan.

A hand-held 125 cm^3 glass atomizer (Fisher Scientific, Pittsburgh, PA) was used to spray coat individual sample disks with an aqueous ethanol zein solution (zein, 10% w/w; glycerol, 2.5% w/w; Myvacet 7-00, acetylated monoglycerides, Eastman Kodak Co., Rochester, NY, 1.0% w/w; 95% ethanol, 86.5% w/w). Coated and uncoated samples received a surface application of sorbic acid by spraying them with a solution of 10% sorbic acid in 95% ethanol. Sorbic acid spraying conditions for a surface application equivalent to 1 mg/cm^2 was determined experimentally as follows: several samples were sprayed for 5, 10, 15, 30, 45, 60 and 90 s with the sorbic acid solution using the same glass sprayer.

TABLE 2.
EFFECT OF HEATING AND STORAGE ON SORBIC ACID STABILITY: BULK APPLIED K-SORBATE

pH[a,b]	%K-sorbate time 00[c]	%K-sorbate time 0[c]	%Remaining K-sorbate[d]	
			Pasteurized	Non-Pasteurized
6.3 ± 0.06 (n = 4)	0.55	0.57	84.7	84.7
6.5 ± 0.03 (n = 4)	0.95	1.06 ± 0.11 (n = 3)	84.7	83.4
6.6 ± 0.02 (n = 4)	1.98	2.10 ± 0.07 (n = 3)	86.0	91.8
6.8 ± 0.04 (n = 4)	4.02	4.24 ± 0.03 (n = 3)	76.8	84.7
all samples			86.1 ± 3.8	83.1 ± 4.2

(a) Includes pasteurized (2 hours in a water bath at 85°C) and non-pasteurized samples.
(b) n = number of replicates
(c) Time 00 and 0 correspond to samples taken before and after pasteurization, respectively.
(d) After 38 days storage at 35°C, 88% RH air and in the dark

A plot of sample sorbic acid concentration versus spraying time indicated that about 17 s were sufficient to achieve the desired preservative surface concentration.

All samples and including controls were stored in the constant RH chamber. Preservative concentration was determined by HPLC as described by Torres *et al.* (1985). *S. aureus* growth response was determined by plating on Brain Heart Infusion agar (BHIA, Difco, Ann Arbor, MI).

RESULTS

The analysis of homogeneous K-sorbate concentration samples (Table 2) showed that initial preservative concentration affected pH of the model system giving values in the 6.3 to 6.8 range. No attempt was made to correct for this pH variation. Heat treatment (2 h at 85°C) resulted in no detectable K-sorbate losses and had no effect on degradation during storage (38 days).

K-sorbate determinations, expressed as percentage of initial concentration (Fig. 1), indicate that losses were less than about 25% after 38 days storage for
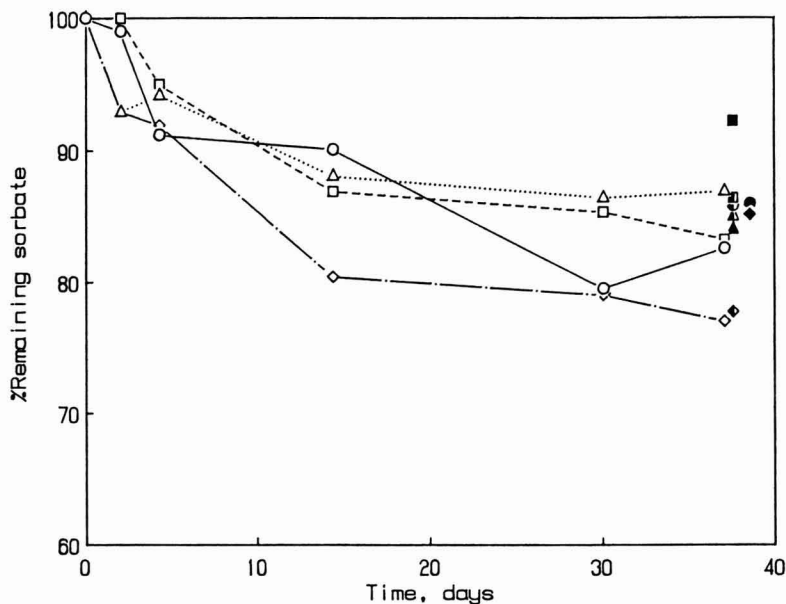
FIG. 1. K-SORBATE ACID DETERMINATIONS AS % INITIAL CONCENTRATIONS
Initial concentrations, % K-sorbate (w/w): 0.57 [O]; 1.06 [◇]; 2.10 [△] and 4.24 [□]. Except for controls, samples were inoculated with $10l^6$ to $10l^7$ cells/cm². Controls were either pasteurized [●, ◇, △, ◻] or not [●, ◆, ▲, ■]. Note that all controls were analyzed on day 37, however, to avoid symbol overlap data points were shifted to the right of Fig. 1.
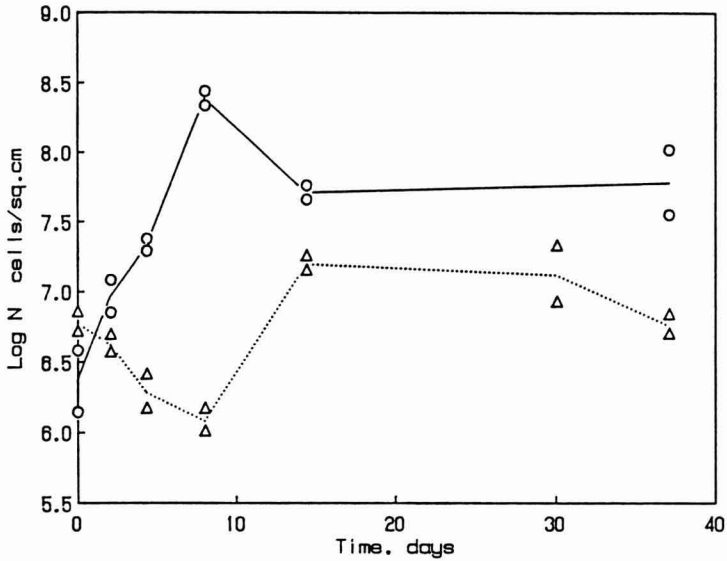
FIG. 2. MICROBIAL GROWTH RESPONSE AT HIGH K-SORBATE CONCENTRATIONS Experiment was run in duplicate. Lines have been drawn through maximum and minimum values. Samples with higher preservative concentrations, inoculated at the same level, showed no viable counts. Initial preservative concentrations, %K-sorbate (w/w): 0.57 (pH = 6.3) [O] and 1.06 (pH = 6.5) [◇].

TABLE 3.
EFFECT OF HEATING AND STORAGE ON SORBIC ACID STABILITY: SURFACE
APPLIED SORBIC ACID

Time, h	0x[a]	1x[a]	3x[a]	Average
2	23.2	18.2	12.0	18.9 ± 6.8, n=5
			(c.v. = 36%)	
19	22.6	26.2	19.1	22.6 ± 5.3, n=6
44	19.7	16.7	16.7	17.7 ± 2.1, n=6
68	11.6	22.5	12.3	15.4 ± 6.1, n=5
118	21.1	21.2	15.3	20.0 ± 3.9, n=5
168	38.7	20.0	14.5	21.5 ± 10.2, n=6
228	15.5	14.5	14.4	14.8 ± 4.0, n=6
All samples				18.6 ± 6.1, n=39
			(c.v. = 33%)	

(a) Uncoated samples [0x] and samples sprayed with zein one time [1x] or three times [3x].

samples that were inoculated with *S. aureus*. These data suggest that there was no concentration effect on degradation rate within the range considered in this study, 0.5 to 4.2% K-sorbate.

Remaining K-sorbate values for uninoculated pasteurized and nonpasteurized controls are also shown in Fig. 1. The comparison of controls and inoculated samples showed that presence of *S. aureus* played no role in K-sorbate degradation (Fig. 1). Furthermore, Fig. 2 shows that significant growth was detected at 0.57% K-sorbate, initial decreases with later growth were observed at 1.06%, while at 2.10 and 4.24% total counts remained below detectable levels (about 1,000 cells/cm^2) during the length of the test (38 days). None of these behaviors had a detectable effect on stability (Fig. 1).

As indicated in Table 3 surface applications showed significant variations in the amount of sorbic acid deposited on each individual piece (2 h, overall c.v. = 36%). An analysis of variance of these data showed no statistically significant differences between uncoated controls (0x) and samples coated with zein one (1x) or three (3x) times. This analysis showed also some effect of storage time on sorbic acid stability. A least significance difference test suggested that this sorbic acid loss had occurred during the first day of storage (Anon. 1985). A similar behavior can be observed in Fig. 1 for samples with homogeneous preservative concentration. The amount of information collected so far is insufficient to identify the sorbic acid degradation kinetics when the preservative is applied on the surface of foods. However, the limited information collected suggests that the surface degradation rate can be expected to be slow and not very different to what has been observed for other application methods and lower concentrations.

CONCLUSION

Sorbic acid is stable under common processing and storage conditions, including microbial growth, preservative concentration variations, mode of application (surface versus homogeneous bulk concentration) and processing conditions (moisture removal by warm air to adjust a_w and heating for two hours in a water bath at 85°C). None of these treatments had a significant effect on its stability. These results were consistent with published findings (Heintze 1976); Bolin *et al*. 1980; Saxby *et al*. 1982).

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POTASSIUM SORBATE PERMEABILITY OF METHYLCELLULOSE AND HYDROXYPROPYL METHYLCELLULOSE MULTI-LAYER FILMS[1]

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ABSTRACT

Spoilage by microorganisms growing on food surfaces is the shelf-life limiting factor for many food products. Previous publications have shown that this shelf-life limitation can be overcome by edible coatings lowering the diffusion rate into the food of antimicrobial agents applied on food surfaces. A permeability cell has been used to evaluate the potassium sorbate barrier properties of polysaccharide based films. In this paper we examine the effect of film formation technique and film formulation on the permeability rate of methyl- and hydroxypropyl methyl cellulose based films. Permeability constant determinations ranging from 10^{-9} to 10^{-11} $(mg/s\ cm^2)(cm)/(mg/cm^3)$ indicate that surface resistance to microbial growth could be enhanced significantly. Scanning electron microscopy examinations showed that films were of uniform thickness. Morphological differences between films were consistent with permeability measurements.

INTRODUCTION

Surface microbial stability is a major determinant of the shelf-life of many food products including refrigerated meats and intermediate moisture foods (IMF) (Vojdani and Torres 1989a,b). During storage and distribution of refrigerated meats, beef, poultry and seafood, nearly all microbial growth occurs on the surface and Gram-negative bacteria are predominant (Ingram and Dainty 1971; Dainty *et al.* 1975; Leistner *et al.* 1981; Anon. 1983). In the case of IMF's, surface condensations caused by temperature fluctuations result in temporary and local increases in surface water activity (a_w). Rapid microbial growth is possible in these localized regions (Torres *et al.* 1985a,b; Torres 1987).

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The solution to surface microbial stability problems investigated in this and previous papers is to combine an edible impermeable coating and an antimicrobial agent such as potassium sorbate. The concentration of the surface-applied preservative is kept high by selecting an edible coating that retards preservative diffusion from the food surface into the food bulk (Torres *et al.* 1985a,b; Torres 1987; Vojdani and Torres 1989a,b). The use of edible coatings for this and other purposes has been recently reviewed by Guilbert (1986).

In a previous study (Vojdani and Torres 1989a), the potassium sorbate barrier properties of films composed of a mixture of fatty acids (FA) and cellulose derivatives, methyl cellulose (MC) and hydroxypropyl methylcellulose (HPMC), were determined using a permeability cell. Edible films with similar compositions have been studied by Fennema and coworkers (Kamper and Fennema 1985, 1984a,b; Kester and Fennema 1986) who have measured their water vapor migration barrier properties.

Methylcellulose films with palmitic, stearic and arachidic acid with a MC:FA = 45:15 ratio showed the following K-sorbate permeability values at 24°C: 1.2×10^{-8}, 8.9×10^{-9} and 4.9×10^{-9} (mg/s cm^2)(cm)/(mg/cm^3), respectively. At 5°C, the same films had the following permeabilities: 4.4×10^{-9}, 3.4×10^{-9} and 2.1×10^{-9} (mg/s cm^2)(cm)/(mg/cm^3), respectively. These values are comparable with those previously measured for zein films (Torres *et al.* 1985a). Microbial challenge tests have shown that zein films controlling surface K-sorbate concentration provide a tenfold increase in surface microbial stability (Torres and Karel 1985).

In this paper we report the effect on permeability values as influenced by the film casting technique. We have also examined films containing hydrogenated palm oil and white beeswax. Electron microscopy techniques were used to examine the morphological characteristics of these films.

MATERIALS AND METHODS

Reagents

Methylcellulose (MC, Methocel A 15-LV, Premium) and hydroxypropyl methylcellulose (HPMC, Methocel F50, Premium) were obtained from Dow Chemical Co., Midland, MI. Polyethylene glycol 400 (PEG), lauric acid (C12, 99-100%), palmitic acid (C16, 99%), stearic acid (C18, 99%), arachidic acid (C20, 99%) were obtained from Sigma Chemical Co., St. Louis, MO. A C16-C18 (1:1, w/w) blend was prepared in the laboratory. Other chemicals used were glycerol (J.T. Baker Chemical, Phillisbury, NJ), white beeswax (Eastman Kodak Co., Rochester, NY), hydrogenated palm oil (Durkee 27, Durkee SCM, Corp., Cleveland, OH) and potassium sorbate (K-sorbate, Monsanto Co., St. Louis, MI). Ethanol (95%, reagent) was obtained from OSU Chemical Stores.

Film Preparation

Double Layer Films. The same technique previously described for single layer films (Kamper and Fennema 1984a; Vojdani and Torres 1989b) was used for the preparation of double layer films. The only difference between the first and the second layer was that the base layer contained no fatty acids. The second layer was cast with a MC or HPMC:fatty acid ratio of 45:20. The first layer was allowed to dry before applying the second layer which was done without changing the thickness setting (0.75 mm) of the thin-layer chromatography spreader.

The objective of this film preparation method was to determine whether improved films could be obtained by reducing the influence of the glass plate on film casting. It should be noted that the overall permeability of these multi-layer films is controlled by the permeability of the second layer which has been shown to have much lower permeability values (Vojdani and Torres 1989a,b).

Coated Films. A base layer containing no fatty acids was prepared as described by Vojdani and Torres (1989b), and then dried and peeled off from the plate. It was then carefully replaced on the glass plate making sure that no air bubbles had formed between the plate and the film. A hot solution of lipids or edible wax (ca. 80°C) was then spread on the base layers using the same warm spreader and a thickness of 0.25 mm. This application method differed from the one used by Kamper and Fennema (1984a) who painted the second layer onto the film.

Embedded Films. In this method the lipid layer was secured between two layers of HPMC. The top and bottom HPMC layers provided protection against cracking during testing.

Film Thickness Measurement

The thickness of films prepared in this study was measured as described by Vojdani and Torres (1989a,b).

Determination of the Permeability of Multi-layer Films

A multi-layer film composed of three sheets of thickness l_1, l_2 and l_3, and permeabilities K_1, K_2 and K_3, respectively, placed in series has under steady state conditions an effective permeability, K_{123}, given by (Karel 1975; Crank 1976; Ashley 1985):

$$l_{123}/K_{123} = l_1/K_1 + l_2/K_2 + l_3/K_3 \qquad (1)$$

where:

K_{123} = permeability of the composite film

$l_{123} = l_1 + l_2 + l_3$

In the case of double layer films, and assuming that the two layers are of equal thickness, the equivalent formula for double layer is:

$$2/K_{12} = 1/K_1 + 1/K_2 \tag{2}$$

Estimations of Enhanced Surface Microbial Stability

The enhanced surface microbial stability period (t, days) can be estimated using the equation derived by Torres *et al.* (1985a):

$$t = 1^2/(86400)\pi f^2 K \tag{3}$$

where:

1 = coating thickness, (cm)

K = permeability coefficient, $(mg/s\ cm^2)(cm)/(mg/cm^3)$

f = a dimensionless factor (= 0.05)

f represents the reduction in surface concentration caused by diffusion into the food bulk. It should be noted that this expression gives only rough estimations that should be confirmed by microbial challenge tests (Torres and Karel 1985).

Electron Microscopy Studies

Electron microscopy was used to evaluate film structure and the distribution of fatty acids in them. Of particular interest were film uniformity and detection of pores. It was also used to confirm film thickness measurements.

The specimens were mounted on aluminum plancets using Avery Spot-O-Glue. The film was sectioned with a sharp razor blade and coated with approximately 100-200A° of 60:40 gold-palladium in a Varian VE-10 vacuum evaporator at a vacuum of 1×10^{-5} torr. The microscopic examination was made using an AMRAY 1000A SEM operated at 20KV at the Electron Microscope Facility, Oregon State University. Images were recorded on Polaroid type 55 positive/negative 4×5 format film. Samples were photographed at effective tilt angles of $+30$ to $+60°$ towards the detector with respect to the incident electron beam.

RESULTS AND DISCUSSION

Selected values obtained from a previous study on the permeability of single layer films have been summarized in Table 1 (Vojdani and Torres 1989b) and include MC and HPMC:FA ratios of 45:15 and 45:20. In the case of the 45:15 ratio we included data at 5 and 24°C.

Permeability Studies

Double Layer Films. Table 2 summarizes our determinations of the permeability values of films cast with the first layer containing no fatty acid while the

TABLE 1.
EFFECT OF COMPOSITION ON THE PERMEABILITY[a] OF SINGLE LAYER FILMS

| | | $Kx10^9$ MC or HPMC:FA 45:15 | | $Kx10^9$ MC or HPMC:FA 45:20 |
		$5^\circ C$	$24^\circ C$	$24^\circ C$
A. MC				
	Control		49	49
			47	42
	Lauric	10	30	22
	(C12:0)	12	27	22
	Palmitic	4.8	12	4.4
	(C16:0)	4.0	12	5.4
	Stearic	3.2	9.4	12
	(C18:0)	3.6	8.4	8.4
	Arachidic	2.1	5.4	---
	(C20:0)	2.0	4.9	---
B. HPMC				
	Control		87	87
			78	78
	Lauric	19	48	28
	(C12:0)	17	43	28
	Pamitic	8.1	20	6.1
	(C16:0)	7.0	21	6.0
	Stearic	5.8	15	24
	(C18:0)	6.5	16	26
	Arachidic	3.5	9.2	---
	(C20:0)	3.9	9.5	---

[a] $(mg/s\ cm^2)(cm)/(mg/cm^3)$

second layer contained various fatty acids at a MC or HPMC:FA = 45:20 ratio. The first layer was cast with the thin layer applicator set at 750 μm which when dry had a thickness of approximately 20 μm. The second layer was cast on the surface of this dry layer without changing the applicator setting. This procedure allowed us to assume that the thickness of each layer was approximately the same. Equation (2) was then used to estimate the permeability of the second layer (K_2) using double layer values (K_{1+2}). As shown by SEM analysis no significant amount of mixing appears to have occurred between individual layers (See section on Electron Microscopy Studies).

The trend previously reported (Vojdani and Torres 1989b), longer chain fatty acids yielding better films, is not quite evident, particularly for HPMC:FA films.

TABLE 2.
EFFECT OF COMPOSITION ON THE PERMEABILITY[a] OF DOUBLE LAYER FILMS
AT 24°C

	K_{1+2}[b] $\times 10^9$	K_1[c] $\times 10^9$	K_2[d] $\times 10^9$	l_{1+2}[e] μm
A. MC				
Control	- - - -	47		26
		44		28
Lauric	22		15	46
	22		15	48
Palmitic	4.4		2.3	54
	6.0		3.2	48
Stearic	11		6.0	54
	12		6.8	54
Arachidic	6.1		3.2	38
	6.3		3.4	38
B. HPMC				
Control	- - -	87		28
		78		26
Lauric	31		19	44
	29		18	42
Palmitic	25		15	38
	28		17	40
Stearic	30		18	46
	28		17	48
Arachidic	11		5.6	44
	12		6.6	42

[a] $(mg/s\ cm^2)\ (cm)\ /\ (mg/cm^3)$
[b] Permeability of double layer film
[c] Permeability of base layer
[d] Permeability of top layer with a MC or HPMC:FA = 45:20 ratio
[e] Thickness of double layer film, μm

The lowest permeability value was observed for MC:palmitic acid films. The reasons for the better values obtained for palmitic acid are not clear and require further studies.

Estimated values (K_2, Table 2) are lower than the equivalent values obtained for single layer films (Table 1, 45:20 data at 24°C). This was due to the single layer film casting difficulties encountered with higher fatty acid concentrations which were not noticed in double layer films. As previously reported arachidic

TABLE 3.
EFFECT OF COMPOSITION AND TEMPERATURE ON THE PERMEABILITY[a]
OF VARIOUS MULTI-LAYER FILMS

	$K_{\times 10^9}$	K_2[b]	l_1[c]	l_{1+2}[d]	l_{1+2+1}[e]
HPMC+Beeswax					
at 24°C	0.045	0.034	30	133	-
	0.058	0.045	25	115	-
HPMC-C18,16-HPMC					
at 24°C	4.2	2.9	21	109	130
	3.4	2.2	22	100	122
at 5°C	1.9	1.2	23	105	128
	1.3	0.85	20	90	110

[a] $(mg/s\ cm^2)\ (cm)\ /\ (mg/cm^3)$
[b] Permeability of the second layer, i.e. bees wax or the C18-C16 mixture
[c] Thickness of the base layer, μm
[d] Thickness of the base and second layer, μm
[e] Thickness of the three layer film, μm

acid single layer films could not be prepared with a 45:20 ratio (Table 1, Vojdani and Torres 1989b). Therefore, a more relevant comparison between single and double layer films is to compare K_2 values at a 45:20 ratio (Table 2) with K values obtained at a 45:15 ratio (Table 1). This comparison shows an improvement for films cast on top of a MC or HPMC base as compared to those cast on glass. This suggests that future studies should examine the effect of food surfaces on the coating properties. Particular attention should be given to surface tension considerations.

Using the approach described by Torres (1987, Table 14.2) it is possible to estimate the effect that double layer films (with permeability values equal to those measured in this study) have on increased microbial stability. For example, if one assumes that it is possible to cast films on a food with the same permeability value (K_2) as reported in Table 2, the surface protection can be predicted to last 2 months for a 0.1 mm film and 7 months for a 0.2 mm thick MC:palmitic acid = 45:20 ratio film.

Coated Films. MC and HPMC films were coated with hydrogenated palm oil and a palmitic-stearic mixture (50% w/w) but resulted in brittle surfaces that could not be tested in the permeability cell. On the other hand, HPMC films coated with beeswax resulted in films with extremely low permeability values. Values reported in Table 3 should only be considered an upper boundary estimate. The permeability cell method became an inconvenient procedure for coatings with permeability values on the order of 10^{-11} $(mg/s\ cm^2)(cm)/(mg/cm^3)$. More-

over, predicted surface protection for such low permeability values indicates that product deterioration would occur by a spoilage mechanism other than surface microbial growth.

Permeability values at other temperatures can be estimated using an Arrhenius model approach. An alternative is the use of the following expression whose validity for several films was demonstrated by Vojdani and Torres (1989a,b):

$$K \, \mu/T = \alpha \qquad (4)$$

where:

K = permeability value
μ = solvent viscosity
T = absolute temperature
α = a constant

Using this approach we estimated that the permeability at 5°C of a HPMC film coated with beeswax would be in the order of 1×10^{-11} (mg/s cm^2)(cm)/ (mg/cm^3), i.e., 2–3 orders of magnitude lower than the values obtained by Torres *et al.* (1985a) for zein films.

Embedded Films. An attempt was made to determine the permeability of pure fatty acid films. A palmitic-stearic acid mixture (50% w/w) was embedded between two layers of HPMC. Samples of the film having the first, the first two and all three layers were used to determine the approximate thickness of each layer needed to estimate the permeability coefficient of the pure fatty acid layer (Table 3). The average K_2 value was 2.6×10^{-9} (mg/s cm^2)(cm)/(mg/cm^3) and should be compared with the higher values obtained for single layer films containing these fatty acids (Table 1).

Kamper and Fennema (1948a) reported that HPMC films coated with hydrogenated palm oil had good moisture barrier properties. Unfortunately, embedded films using hydrogenated palm oil as the middle layer cracked easily and could not be tested in our permeability cell.

Electron Microscopy Studies

Morphological differences between individual film compositions and casting techniques were examined by electron microscopy (Fig. 1–3). Figure 1 shows the presence of fatty acid crystals on the film surface. As shown in Fig. 2, films were of uniform thickness and consistent with the values measured with a Best Test Indicator (Vojdani and Torres 1989a,b). None of the films examined showed the presence of cracks or pores. The thickness differences between films with different composition were expected (Vojdani and Torres 1989b). Cross sections of thicker films, typically beeswax coated and multiple layer films were more difficult to prepare and show that tearing occurred during cutting. No major
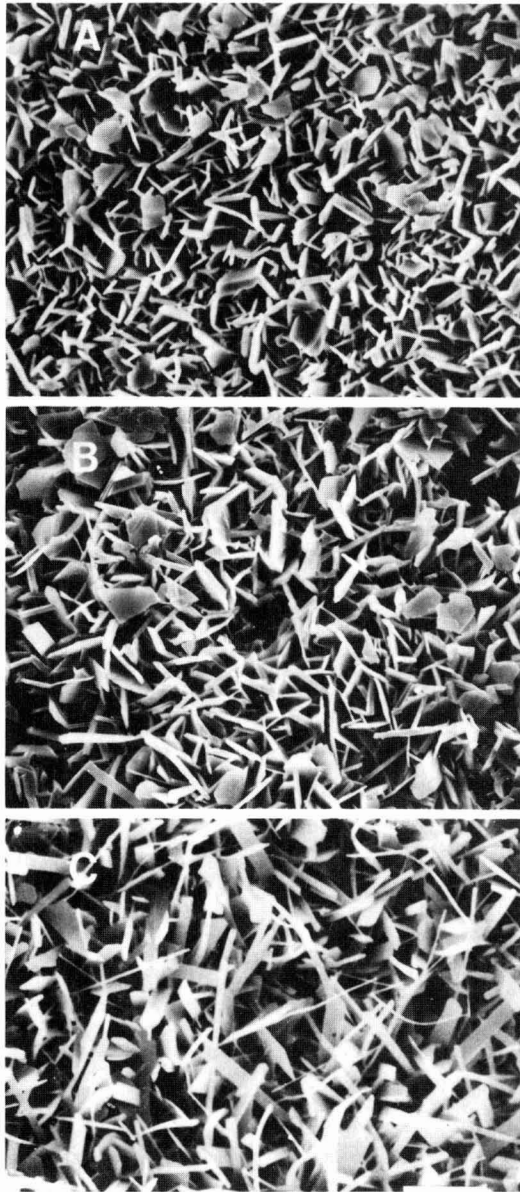
FIG. 1. ELECTRON MICROSCOPY PHOTOMICROGRAPHS OF THE SURFACE OF SINGLE
LAYER FILMS (bar = 10 μm)
a. CE:palmitic acid = 45:15
b. CE:stearic acid = 45:15
c. CE:arachidic acid = 45:15
See text for further details.
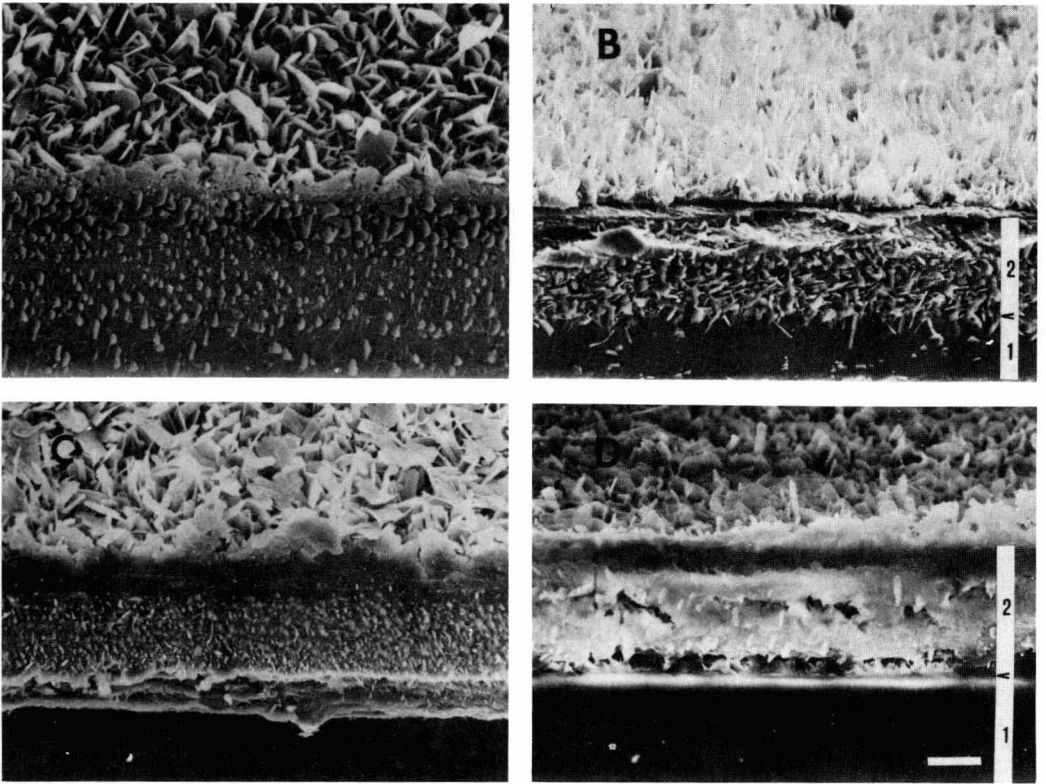
FIG. 2. ELECTRON MICROSCOPY PHOTOMICROGRAPHS OF CROSS-SECTIONS OF
EDIBLE COATING FILMS (bar = 10 μm)
a. MC:palmitic acid = 45:15, single layer film
b. HPMC:arachidic acid = 45:15, double layer film
c. MC:stearic acid = 45:15, single layer film
d. HPMC:stearic acid = 45:15, double layer film
Numbers 1,2,3 indicate layer application order. See text for further details.

differences were seen between MC and HPMC based films, thus the following
discussion will concentrate on the effect of the fatty acid component.

Single Layer Films. Figure 1 shows top view photomicrographs of the surface
of single layer films containing palmitic, stearic and arachidic acid at a MC or
HPMC:FA = 45:15 ratio. Lauric acid films are not shown because they could
not withstand the electron beam.

Although single layer films were cast from an homogeneous mixture (Vojdani
and Torres 1989b) fatty acids tended to migrate to the surface and formed mostly
vertical crystals. As noted by Kamper and Fennema (1984a) the fatty acids orient
themselves at the air-film solution interface during film formation.
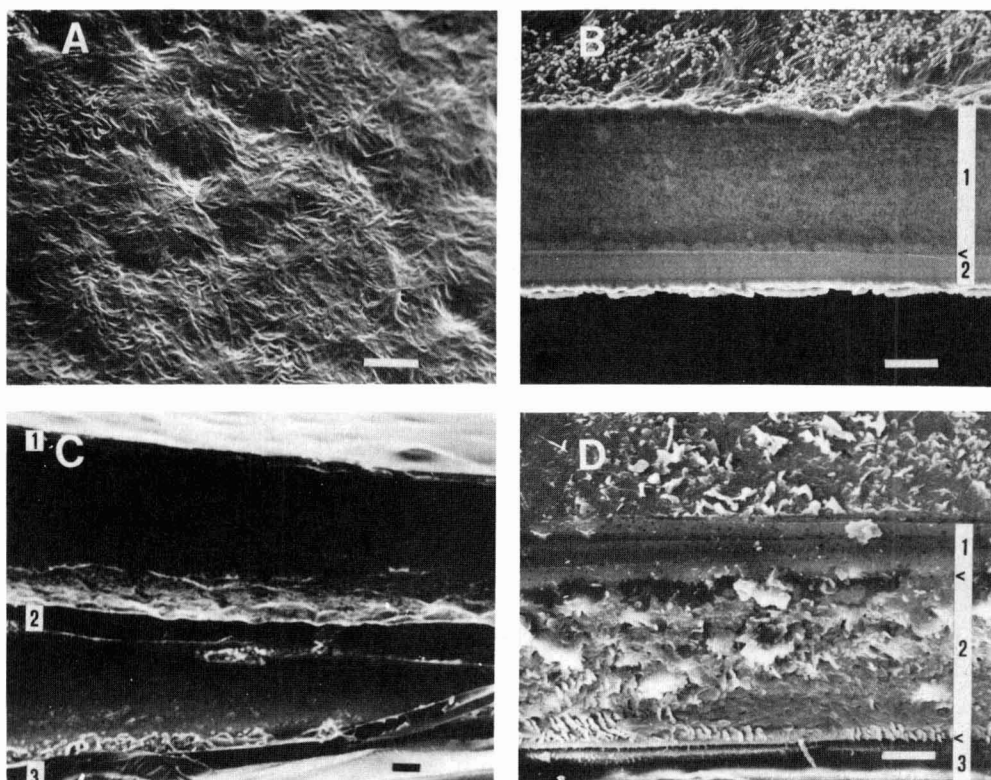
FIG. 3. ELECTRON MICROSCOPY PHOTOMICROGRAPHS OF MULTI-LAYER FILMS
(bar = 10 μm)
a. surface of bees wax coated HPMC film
b. cross-section of bees wax coated HPMC film
c. cross-section of hydrogenated palm oil embedded between two HPMC layers
d. cross-section of a C18-C16 mixture embedded between two HPMC layers
Numbers 1,2,3 indicate layer application order. See text for further details.

Differences can also be seen between the fatty acid crystalline structures observed. Both palmitic and stearic acid formed on the film surface polygonal flat crystals, however, stearic acid crystals were larger. On the other hand, arachidic acid formed large rectangular, flat and ribbon-like crystals.

Surface spots with no crystals were observed in films with stearic acid, which were more frequent in the case of arachidic acid but were not seen with palmitic acid. This observation seems to be consistent with the apparent fatty acid distribution differences within the film itself. The cross section shown in Fig. 2a suggests that in films with palmitic acid there is a bottom to top surface increase in the distribution of fatty acids which is less pronounced for stearic (Fig. 2c)

and arachidic acid (Fig. 2b). This suggests that palmitic acid had fewer difficulties in reaching the film surface as compared to stearic and arachidic acid. Further studies are required to confirm these preliminary observations.

Double Layer Films. No major differences were noted on the surface of double layer films. The most significant observation was that electron microscopy studies provided support to our assumption that each layer had the same thickness and that no mixing occurred between individual layers (Fig. 2b and 2d). Note also the lack of features of the cellulose ether layer.

Coated Films. Figures 3a and 3b show a HPMC film coated with beeswax. In this case no crystalline structures were observed and the beeswax formed a very compact layer without voids. Kamper and Fennema (1984a) had observed that films coated with solid beeswax were very effective barriers to the transfer of water vapor, but that these films were extremely brittle. We found that our preparation method yielded films that were not only excellent K-sorbate permeability barriers but were also very flexible. Beeswax is known to provide excellent moisture transfer control (Watters and Brekke 1961).

Embedded Films. Figure 3d shows a palmitic-stearic acid mixture embedded between two layers of HPMC of approximately equal thickness. During casting some of the fatty acids leached out as shown by the presence of crystals on the film surface.

Figure 3c confirms our previous observation that hydrogenated palm oil embedded between HPMC layers formed poor films. This photomicrograph shows that individual layers tended to separate.

CONCLUSIONS

Permeability determinations summarized in Tables 1–3 are in the range of 10^{-9} to 10^{-11} (mg/s cm^2)(cm)/(mg/cm^3). The lowest values correspond to HPMC-beeswax double layer films. Single layer films containing fatty acids could be used to extend the relatively short shelf-life of refrigerated products whose stability is limited by surface microbial growth. Estimation of the surface protection period achieved by the use of double layer films with beeswax indicate that these films could extend the shelf-life of intermediate moisture foods stored at room temperature.

Scanning electron microscopy studies suggest a correlation between fatty acid distribution and permeability values. For example, single layer films containing palmitic acid were found to have lower than expected permeability values which correlate with the better surface fatty acid coverage noted in photomicrographs of these films. In the case of double layer films using beeswax a uniform surface coverage was also noted.

Edible bilayer films of the kind reported here and in previous papers (Vojdani and Torres 1989a,b) appear flexible and strong and should be able to withstand reasonable mechanical stresses during food distribution. Future studies will determine the diffusion of sorbate in food model systems coated with these films. The effectiveness of diffusion control should also be evaluated using microbial challenge tests.

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INFLUENCE OF GLUCONIC ACID ON THERMAL PROCESS-
ING REQUIREMENTS FOR CANNED WHOLE PEELED
TOMATOES

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ABSTRACT

Methods of detecting the activities of endogenous tomato pectin esterase and polygalacturonase were developed and used to measure heat resistance parameters for enzymes in tomato homogenates. Addition of 0.44% gluconic acid reduced heat resistance of pectin esterase which is responsible for gel formation in canned whole peeled tomatoes. However, heat inactivation of tomato polygalacturonase, which is responsible for general degradation of texture/consistency in tomato products, was not significantly affected by addition of up to 1.65% by wt gluconic acid. Pectic enzymes in canned whole peeled tomatoes packed in juice in 303 × 406 cans were inactivated in 35 min at 98.9 and 101.7°C, 26 min at 110.0°C or 25 min at 118.3°C.

INTRODUCTION

Tomato products are acid foods (pH < 4.6) that require relatively mild heating to control spoilage microorganisms and enzyme systems capable of degrading the finished product. Further acidification with organic acids to reduce heat resistance of microorganisms is commercial practice (Judge and Sons 1986). By acidifying to pH < 4.3, the problem of the most heat resistant flat-sour microorganisms is eliminated (York *et al.* 1975). For acidified tomatoes, therefore, severity of heat processing may be designed to inactivate pectic enzymes which are the most heat resistant enzymes in tomatoes and are responsible for texture

degradation in the canned product (Schwimmer 1981). There is some evidence that acids can either decrease the heat resistance of pectic enzymes (Moy *et al.* 1978), or inhibit their activity (Wagner and Miers 1967).

Citric acid, a component of tomatoes, is the most commonly used acidulant. However, tartness of citric acid limits the amount that can be added without adversely affecting product flavor; at palatable levels, citric acid addition is not sufficient to reduce heat resistance of pectic enzymes. Considering other GRAS organic acids, gluconic acid has the potential to reduce pH below 4.0 without detracting from sensory qualities. The purpose of this study is to evaluate the influence of commercially feasible levels of gluconic acid on heat resistance of pectic enzymes in tomatoes, on process requirements and product quality in canned whole peeled tomatoes.

MATERIALS AND METHODS

Assays Development

Two major pectin-degrading enzymes in tomatoes are pectin esterase (PE), and polygalacturonase (PG). Although enzyme properties can be affected, to avoid side reactions during assaying of enzyme activity, the enzymes must be extracted and at least partially purified (Whitaker 1972). To prevent any alteration of the enzyme functionality in this study, the endogeneous tomato enzymes were retained in their natural environment. Activity was assayed by means of the characteristic enzyme action on a substrate. More specifically, since PE activity in canned tomatoes causes gel formation, activity of PE was assayed by observing gel formation in standard pectin solutions; and since action of PG causes sharp decrease in tomato serum viscosity, PG activity was evaluated by measuring changes in viscosity of standard pectin solutions. Activity of PE was inhibited by reduction of pH when assaying PG activity.

Substrate. Substrate solution was made with 3% by weight/volume slow set citrus pectin (Sunkist Growers, Inc., Corona, CA) in deionized water. The granules were dissolved by adding 10 mL of 10% HCl/L pectin suspension, and heating the mixture to near boiling, or until the granules dissolved. The solution was filtered through four layers of gauze to remove any undissolved clumps of pectin. To retard microbial activity, 0.1% by weight/volume sodium benzoate was added. The solution, pH 2.2 ± 0.1, was refrigerated until needed. Other commercially available pectins can be screened for suitability as substrates by measuring changes in viscosities before and after incubation with crushed raw tomato sample. If viscosity of 3% pectin solution drops from approximately five to near one centipoise, that pectin can be considered a suitable substrate.

Sample Preparation. Processing tomatoes were crushed with 5% by wt NaCl at high speed in a blender. The whole homogenates, containing both seeds and

```
┌─────────────────────────┐
│   10# WHOLE TOMATOES    │
└─────────────────────────┘
            │
┌─────────────────────────┐
│ CRUSH WITH 5 % Wt/Wt NaCl │
└─────────────────────────┘
            │
┌─────────────────────────┐
│   DIVIDE HOMOGENATE     │
└─────────────────────────┘
            │
┌─────────────────────────┐
│ ADD % Wt/Wt GLUCONIC ACID │
└─────────────────────────┘
            │
┌─────────────────────────┐
│ FILL TUBES WITH 4 ml SAMPLE │
└─────────────────────────┘
            │
┌─────────────────────────┐
│      VACUUM SEAL        │
└─────────────────────────┘
            │
┌─────────────────────────────┐
│ HEAT DESIRED TIME/TEMPERATURE │
└─────────────────────────────┘
            │
       ┌────────┐
       │  COOL  │
       └────────┘
```

ASSAY FOR PECTINESTERASE	ASSAY FOR POLYGALACTURONASE
2 mL TOMATO HOMOGENATE 25 mL 3 % PECTIN SOLUTION 15 mL ACETATE BUFFER	2 mL TOMATO HOMOGENATE 25 mL 3 % PECTIN SOLUTION
INCUBATE 30 Min. @ 37°C	INCUBATE W/AGITATION 2 Hr. @ 37°C
BOIL 11 MIN.	BOIL 11 MIN.
REFRIGERATE OVERNIGHT	COOL TO 25°C
OBSERVE	FILTER
CENTRIFUGE	MEASURE VISCOSITY
OBSERVE FOR PRESENCE OF GEL	

FIG. 1. SCHEMATIC ILLUSTRATION OF SAMPLE PREPARATION FOR EVALUATING
HEAT RESISTANCE OF PECTIN ESTERASE (PE) AND
POLYGALACTURONASE (PG) IN WHOLE TOMATO HOMOGENATE
For PE, end point determinations were made (enzyme either active or not active). For PG,
D values (times needed to reduce activity by 90%) were measured.

skins, were divided into six containers to which different levels (0 to 1.65% by wt) of gluconic acid were added. The samples were held on ice or refrigerated until used. A flow diagram of sample preparation and assay procedures is given in Fig. 1.

Pectin Esterase. For estimating pectin esterase (PE) activity, 2 mL of tomato homogenate were added to a standard pectin mixture consisting of 25 mL 3% pectin and 15 mL 0.5M sodium acetate buffer. The pH of the mixture was >

4.5. Samples were incubated 30 min at 37°C, heat inactivated (11 min at 100°C), and chilled up to 24 h. Appearance of a white haze indicated PE activity. When the samples were centrifuged 30 min at 6000 rpm and filtered, the demethylated pectin separated as a white gel residue. Thus, the presence or absence of PE was identified.

Polygalacturonase. Two mL of tomato homogenate were mixed with 25 mL pectin solution in covered 125 mL flasks. A number of incubation times and temperatures (between 25 and 50°C) (Pozsar-Hajnal and Polacsek-Racz 1975; Liu and Luh 1980) were tested. Best reproducibility was achieved with 2 h incubation at 37°C with constant agitation. To stop enzyme action, the mixtures were heated 11 min in boiling water and cooled to room temperature (25°C). Cooled samples were filtered through Whatman No. 4 filter paper, then through a glass fiber filter (GF Type A-E, Gelman Sci. Inc., Ann Arbor, MI), while loading each sample into a size 100 Ostwald-Cannon-Fenske viscometer tube
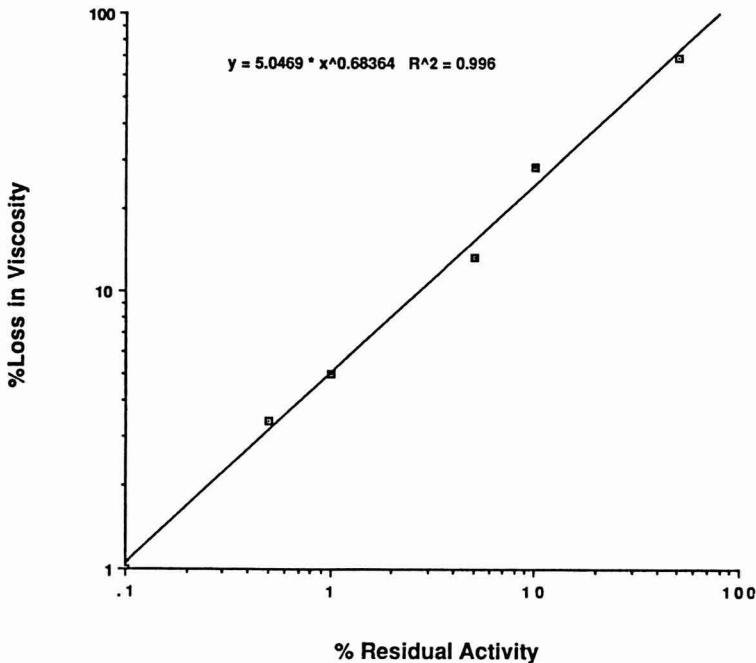

FIG. 2. REPRESENTATIVE STANDARD CURVE FOR % PG ACTIVITY MEASURED IN
TERMS OF LOSS IN SERUM VISCOSITY IN 3% PECTIN SOLUTION
PG activity curves were not affected by addition of acid, but varied with different sources and cultivars.

(Ind. Res. Glassware, Ltd., Roselle, NJ). Flow times were measured in a 30 ± 0.1°C water bath. Viscosity values were calculated as follows:

Viscosity (cp) = flowtime (s) × k (flow tube calibration constant)

Duplicate flow times were measured for each sample. With the same pectin solution, reproducibility of viscosities was ± 0.01 cp.

Standard activity curves such as the one shown in Fig. 2 for PG were established using respective raw tomato homogenates diluted in known proportions (v/v %) with heat inactivated tomato homogenate. The latter homogenate was heated 30 min in boiling water and was assumed to have no enzyme activity. Using the standard activity curves, residual enzyme activity (%) in samples was estimated from % loss of viscosity. Paired tests for PG enzyme activity showed significant differences for varieties but not for the amounts of gluconic acid added.

Heat Resistance Study

For each acidification level, 4 mL of homogenate were placed into 1.0×7.5 cm glass tubes. The tubes were vacuum capped and heated in an agitated constant temperature (± 0.1°C) water bath. Heat resistance was measured at 6 to 12 temperatures in the 53.3–87.1°C range. Heating times, inversely related to temperature, were between one and 70 min. At the end of heating, the tubes were cooled in ice water, and 2 mL of the heated material was transferred to each assay reaction mixture. For PE inactivation, thermal destruction times were calculated using standard TDT procedures (NCA 1968), i.e., heating the samples at progressively longer times until an endpoint time beyond which no enzyme activity was observed in the assay. For PG, % losses in viscosity of pectin solution were converted to % activity values using standard curves for the homogenates, such as shown in Fig. 2. D values were obtained from correlations of log percent activity remaining versus time.

Canned Samples

Enzyme inactivation and process requirements for canned whole peeled tomatoes in 303×406 cans were established using the canned product.

Laboratory Test Packs. Laboratory test packs were prepared as follows: Tomatoes were dipped in boiling water for 20 s, then cooled in running tap water. The tomatoes were hand peeled and packed into 303×406 inside enameled cans. Tomato fill weights were changed with processing temperature: 290.7 − 334 g at 215°F (101.7°C), 331.5 − 385 g at 230°F (110°C), and 325.1 − 388.5 g at 245°F (118.3°C) to simulate commercial practice; higher fill weights were needed at higher processing temperatures to meet drained weight requirements

TABLE 1.
PROCESSING CONDITIONS AND MAXIMUM CAN CENTER TEMPERATURES (CT)
ACHIEVED IN LABORATORY TEST PACKS OF CANNED WHOLE PEELED
TOMATOES IN 303 × 406 CANS. MAXIMUM TEMPERATURES ARE AVERAGES
IN THREE CANS.

Process Time (min)	Process Temperature					
	215°F (101.7°C)		230°F (110°C)		245°F (118.3°C)	
	reel speed (rpm)	max CT (°F)	reel speed (rpm)	max CT (°F)	reel speed (rpm)	max CT (°F)
10	---	---	3.8	153.0 ± 11.3	4.0	188.0 ± 33.9
15	---	---	3.5	164.5 ± 43.1	3.5	207.5 ± 6.4
20	3.5	179.5 ± 14.8	2.9	185.7 ± 18.2	3.0	216.5 ± 12.0
25	2.5	196.3 ± 8.1	---	---	2.5	216.5 ± 16.3
26	---	---	2.4	194.0 ± 1.4	---	---
30	2.5	212.3 ± 3.0	2.4	196.0 ± 1.4	2.4	244.0 ± 2.8
35	1.7	197.2 ± 20.2	---	---	---	---
41	1.7	210.7 ± 4.0	---	---	---	---

(USDA 1964). Tomato count per can averaged 5 ± 1. To each can, 40 grains of sodium chloride were added. The tomatoes were covered with tomato juice which contained gluconic acid at 0 to 1.65% by juice weight. The cans were sealed under 15 in. (381 mm Hg) vacuum and processed in a Steritort cooker. In each retort load, heat penetration at the can center was measured in three cans. The thermocouples were initially located inside tomatoes. Processing conditions and maximum can center temperatures achieved are given in Table 1. Variability in can center temperatures was probably due to tomatoes breaking or slipping off the thermocouples as the cans were rotating. This made determinations of slowest heating points unreliable. Reel speeds were chosen to reflect commercial cooker operation. For each retort time-temperature combination, five cans were packed and analyzed at each gluconic acid level.

Commercial Test Packs. Commercial test packs of canned tomatoes were prepared and packed on-line in a commercial plant. Samples with 0.3% citric acid in the juice were taken from regular production. Experimental samples containing gluconic acid or no acid were processed in a separate rotary pressure cooker. Times, temperatures and cooker speeds are given in Table 2. Can center temperature was measured by puncturing a hot can and inserting a metal thermometer. In the 1985 tomato season, no attempts were made to control either

TABLE 2.
PROCESSING CONDITIONS AND CAN CENTER TEMPERATURES ACHIEVED AT
THE END OF HEATING IN COMMERCIAL TEST PACKS OF CANNED WHOLE
PEELED TOMATOES IN 303 × 406 CANS, PROCESSED AT 210°F (98.9°C)
IN 1985 SEASON. AVERAGES ARE OF EIGHT CANS AT EACH TIME.

Process Time (min)	Cooker Reel Speed (rpm)	Cooker Speed (cans/min)	Can Center Temperature (°F)
20	3.5	174	192.5 ± 5.0
25	2.8	135	194.2 ± 2.0
30	2.3	116	194.5 ± 2.6
35	2.0	100	200.4 ± 2.4
42	1.7	85	201.6 ± 1.3

tomato wholeness or fill weights. In 1986, only 4–6 whole tomatoes per can were used and fill weights were controlled at 313 ± 29 g. Twelve cans per variable were evaluated in this part of the study.

Microbial Activity. Cans of tomatoes were aseptically stab-sampled with a flamed, inverted 2 mL pipette, to avoid potential contamination during blending can contents. Triplicate tubes of 10 mL Acid Product Test (APT) broth (0.75% yeast extract, 1% peptone and 1% invert sugar) were inoculated with 1 mL sample each and incubated at 30°C. Development of cloudiness in the clear broth indicated microbial growth. The formulation was specific for vegetative organisms (lactics, yeasts, etc.) that would grow in the tomato material. Growth was confirmed microscopically.

Enzyme Activity. Cans of whole tomatoes were again stab-sampled in triplicate. Evaluations were done between the second and eighth month of storage. Samples containing tomato flesh and locular material, including seeds, were incubated in the pectin reaction mixture. Pectin solution viscosities were measured to assess PG activity. Apparent enzyme activity was confirmed by comparing decreases in viscosity after incubating the pectin solutions with selected samples before and after the samples were heated 10 min in boiling water.

Physical and Chemical Analyses. Wholeness and drained weight were measured according to U.S. Standards for Grades of canned whole peeled tomatoes (USDA 1964). The method of sampling left characteristic holes which neither detracted from apparent wholeness of tomatoes nor affected comparable drained weights. Juice color was evaluated with a Gardner XL 23 tristimulus colorimeter (Gardner Laboratory, Inc., Bethesda, MD) standardized with D33C-1131 red

tile. Sample pH was measured using an Orion combination glass body electrode and Model 701A digital IONALYZER (Orion Research Inc., Cambridge, MA).

RESULTS AND DISCUSSION

Heat inactivation time for enzymes is analogous to the thermal death time (or F value) used for microorganisms. It is an end-point determination which defines how long the food must be heated at a given temperature so that enzyme activity is no longer observed. Heat inactivation time can be expressed as an F value in terms of D values at a specified temperature (T):

$$F_T^z = D_T^z (\log a - \log b)$$

where "a" describes the initial activity (100%) and "b" the activity which survives the given heat treatment. In commercial processing, "b" must be small enough that the "surviving" enzyme fraction will not cause problems in the processed food. The D value represents time needed at temperature T to destroy 90% of the enzyme activity. D is an exponential function of temperature:

$$D_T = D_{T_{ref}} 10^{\frac{T_{ref} - T}{z}}$$

and is used to define parameter z. $D_{T_{ref}}$ is the D value at reference temperature T_{ref}.

In the present experiments, the assay for PE was an end point determination; it measured only presence or absence of active PE. Therefore, this assay was useful for directly obtaining F values required for inactivating PE. The z value was obtained from the negative reciprocal of the regression coefficient determined for temperature of heating vs log F. The viscosity test method for PG was adequately sensitive to reproducibly detect about 1% loss in viscosity (or 0.1% of the original PG activity). Heating times to achieve this level of destruction could be assumed to be endpoints, or F values. However, since the method was capable of measuring viscosity losses over the full range of 100% to 0.1% enzyme activity, it was suitable for determining D values. D values could then be used to establish F values for any desired level of PG inactivation. The negative reciprocal of the regression coefficient from temperature vs log D yielded the z value.

Heat Resistance of Pectic Enzymes

Pectin Esterase (PE). Pectin esterase is known for firming canned tomatoes and for causing undesirable gel formation in the covering juice. The optimum

pH for plant PE is slightly alkaline, pH 7-9, whereas PE of fungal origin can tolerate a broader range of pH, with most optima falling on the acid side (Schwimmer 1981).

Up to 0.33% gluconic acid addition (pH 3.84 ± 0.08) did not reduce the heat resistance of PE in tomato homogenates. However, above 0.44% gluconic acid levels (pH 3.76 ± 0.08), the heat inactivation time at 82°C was decreased from 51 s to 14 s, and z was decreased from 13°C to 9°C (as compared to $\leq 0.33\%$ gluconic acid).

Extracted tomato enzyme has been reported to have an inactivation time of 15 s at 82°C (Garces 1963; Luh and Daoud 1971). In crushed tomatoes in this work, PE was inactivated in a longer time, 51 s at 82°C, with 0-0.33% gluconic acid added. At higher gluconic acid levels (>0.44%), inactivation time of 14 s was closer to the 15 s literature value for the extracted enzyme. However, the z values for endogenous enzymes (9 and 13°C) in this work were very different from z values of $60.8 - 65.7$°C reported for the extracted enzyme (Garces 1963). Removal of enzymes from their natural environment may be the primary cause for the observed differences.

McColloch et al. (1952) reported enhanced enzyme activity between 50–60°C for the natural enzyme in the tissue. Our results support their finding; enzyme inactivation was not observed in 50 min at 61°C. The enhanced PE enzyme activity at elevated temperatures, before protein denaturation, could cause problems in tomato canning operations. PE could attack pectins as tomatoes heat through heat activation and marginal inactivation temperatures. Addition of gluconic acid could help with the problem in two ways; first, the enzyme activity should be reduced by addition of the acid (Moy et al. 1978) and, second, at levels of 0.44% or above, gluconic acid reduces the heat resistance, thereby enhancing enzyme destruction. Thus, formation of gel in canned whole peeled tomatoes may be controlled. PE enzyme regeneration was not observed.

Polygalacturonases (PG). Unlike PE, which de-esterifies pectins, tomato PGs can split both internal and penultimate linkages, but will not cleave digalacturonic acid. Cleavage of internal linkages results in a sharp drop in viscosity. In published works (Garces 1963; Luh and Daoud 1971; Moshrefi and Luh 1984), activity of PG has been measured primarily using a reaction which depends on the reducing value of the pectic substance. The reducing value increases with enzyme action, since at each cleavage site on the pectin molecule, additional reducing groups become available for the reaction. To avoid interference from other reactions, the enzyme has to be extracted and partially purified. However, removal of enzymes from their natural surroundings could affect both activity and heat resistance. Therefore, it was important in this work to develop a method by which enzyme activity could be estimated without isolating the enzyme. A procedure for quantifying enzyme action as a function of viscosity was developed.

Several substrates, including various tomato products, were tested. With tomato products, pectin concentration and serum extraction were not reproducible, and viscosity values were inherently variable. Suitable substrates with which reproducible information could be gained were either slow or rapid set citrus pectins

TABLE 3.
HEAT RESISTANCE OF PG IN NATURAL AND pH MODIFIED WHOLE TOMATO
HOMOGENATES (INCLUDING SEEDS AND SKINS)

Gluconic Acid	Coefficient of Determination[a] R^2	z value	D values		
			70°C	82°C	104°C[b]
(%)	(%)	(°C)	(min)	(min)	(sec)
Var. UC204 (lot 1; n=6[c])					
0	99.8	11.3	20.5	1.4	1.2
0.28	98.6	11.5	16.9	1.5	1.1
0.55	98.7	11.8	15.6	1.5	1.2
0.82	96.6	12.3	15.0	1.6	1.6
1.10	95.4	12.8	13.5	1.6	1.8
1.38	93.0	12.8	11.0	1.3	1.4
1.65	91.3	14.1	10.9	1.5	2.6
Var. UC204 (lot 2; n=6)					
0	97.6	12.5	25.0	2.8	2.9
0.22	96.0	12.2	23.8	2.5	2.3
0.33	96.8	12.3	24.8	2.6	2.6
0.44	96.5	12.2	25.5	2.7	2.6
0.55	95.9	12.0	23.6	2.4	2.1
0.82	95.5	11.7	27.4	2.6	2.0
1.10	97.4	12.5	25.0	2.8	2.9
Mixed lot (UC204, UC82, Diego, Murietta n=4-6)					
0	97.7	17.8	14.0	3.0	10.3
0.22	95.0	17.7	13.5	2.8	9.7
0.33	97.6	19.1	14.1	3.1	13.1
0.44	97.3	19.0	12.6	2.9	12.3
0.55	96.3	20.9	11.0	2.9	15.7
0.82	97.9	19.3	13.1	3.1	13.7
1.10	96.9	21.4	12.4	3.3	19.4
Fresh market mix (n=12)					
0	85.2	16.1	8.9	1.6	4.1
0.22	84.2	16.2	8.7	1.6	4.2
0.33	84.7	16.0	9.2	1.6	4.1
0.44	84.5	16.4	9.0	1.7	4.6
0.55	86.0	15.9	9.5	1.7	4.1
0.82	84.9	16.3	9.3	1.7	4.6
1.10	81.4	16.1	11.0	2.0	5.7

[a]R^2 of correlations of log D value vs temperature.
[b]Extrapolated reference values for comparison with published data.
[c]n = Number of temperatures at which D value was assessed for each gluconic acid level.

(without sugar). Reaction mixtures were maintained at pH 2.5 ± 0.1 to inhibit PE activity. The recommended sodium (Schwimmer 1981) was provided by the 0.1% sodium benzoate present in the pectin solution and the 5% NaCl used in preparing the homogenate.

Investigation of PG heat resistance was initiated based on results available on extracted enzymes: heat destruction time (F value) of PG has been indicated to be between 20 and 30 min at 60°C, or 15 s at 104°C, with z values ranging between 29.2 and 38.4°C (Garces 1963; Luh and Daoud 1971). As with PE, heat resistance of PG in the whole homogenate was different. For example, at 61°C, 90% inactivation (D) values were 24.8 and 69.0 min for the 1.65% gluconic acid and natural preparations, respectively. At 61°C, heat destruction of PG was expected based on the values reported by others, but in this work, it was not observed with heating up to 50 min. Heat inactivation parameters (D, z) obtained at selected temperatures are given in Table 3. D values at 104°C were calculated from regression equations and added as a basis of comparison with published values.

In one test using a mixture of tomato cultivars, heat resistance of PG was higher at higher temperatures when compared to results on single varieties. Since commercial tomato varieties are mixed for processing, analysis of the data concurs with observations of sporadic enzyme activity in tomato concentrates. Serum separation, an indication of enzyme activity, has been experienced in commercial tomato paste even after heat treatments of 45 s at 220°F (104°C).

Heat inactivation of PG, in general, did not respond to addition of gluconic acid. When Wagner and Miers (1967) studied the inhibition of tomato enzymes with additives, they found that PE and PG could be inhibited with strong acids that lowered pH of the tomato macerate to pH < 1.9. Enzyme inhibition in their work appeared to be a function of pH, and not of the type of acid used. Gluconic acid is a relatively weak acid which cannot achieve PG inhibition by adequately lowering the pH and apparently it did not demonstrate any functional site inhibition of pectic enzymes. Minimum homogenate pH with gluconic acid was 3.22 in this study.

The results show that heat resistance of PG varied with cultivars tested. Natural differences in enzyme concentration, activity and specificity, are assumed to be responsible. Pectic enzymes from microbial sources were unlikely in this study because only whole and uninfected tomatoes were used and preservative was added to the reaction mixture. Regeneration of PG activity after heat treatment was not observed.

Evaluation of Canned Samples

Since there were no definitive end point heating times for PG, final process requirements were determined in both laboratory and commercial test packs of canned tomatoes.

TABLE 4.
VISCOSITY TESTS TO DETERMINE POLYGALACTURONASE ACTIVITY IN
LABORATORY CANNED TOMATOES. VISCOSITIES (CP) OF PECTIN REACTION
MIXTURES INOCULATED WITH LABORATORY TEST PACK TOMATOES (AVG. ±
ONE STANDARD DEVIATION). SAMPLES WITHIN ONE ACID LEVEL AND PROCESS
TEMPERATURE WERE REACTED WITH THE SAME STANDARD PECTIN SOLUTION.

Heating Time[a]	Reel Speed	% Gluconic acid in cover juice					
(min)	(rpm)	0.0 (control)	0.55	0.82	1.10	1.38	1.65
		Process @ 215°F (101.7°C)					
20	3.5	3.46±0.05*	2.50±0.01*	3.52±0.08*	3.49±0.05*	3.56±0.14*	3.50±0.01*
25	2.5	3.59±0.02	3.52±0.03*	3.47±0.04*	3.53±0.06*	3.58±0.06	3.54±0.04*
30	2.5	3.58±0.05	3.62±0.08	3.59±0.01	3.55±0.03*	3.57±0.02	3.58±0.02*
35	1.7	3.63±0.03	3.63±0.04	3.59±0.05	3.61±0.06	3.60±0.04	3.64±0.02
41	1.7	3.61±0.06	3.63±0.03	3.63±0.02	3.67±0.04	3.62±0.03	3.66±0.04
		Process @ 230°F (110°C)					
10	3.8	[2.82±0.10]*	2.87±0.12*	[2.84±0.07]*	[2.91±0.08]*	2.90±0.10*	2.91±0.04*
15	3.5	[2.97±0.06]	2.97±0.03	2.93±0.04*	2.94±0.09*	3.00±0.02	[2.94±0.02]
20	2.9	[2.96±0.04]	2.99±0.03	2.93±0.03*	3.02±0.02	2.96±0.06	[2.93±0.06]*
26	2.4	2.99±0.02	2.97±0.01	2.98±0.03	3.03±0.06	2.98±0.04	2.98±0.03
30	2.4	2.96±0.05	2.97±0.04	2.97±0.04	2.98±0.02	3.01±0.01	2.96±0.03
		Process @ 245°F (118.3°C)					
10	4.0	3.08±0.06*	3.06±0.10*	3.05±0.06*	3.04±0.02*	3.02±0.06*	3.00±0.03*
15	3.5	3.04±0.02*	3.14±0.04*	3.09±0.07*	3.08±0.03*	3.07±0.06*	3.07±0.01*
20	3.0	3.13±0.03*	3.13±0.02*	3.14±0.01	3.10±0.02*	3.13±0.04	3.11±0.02*
25	2.5	3.17±0.03	3.21±0.08	3.15±0.01	3.19±0.02	3.17±0.03	3.16±0.04
30	2.4	3.20±0.08	3.18±0.02	3.17±0.06	3.16±0.03	3.18±0.06	3.19±0.04

[a]Followed by 28 min cooling.
*Averages include viscosity values indicating >0.1% enzyme activity in one or more of the cans tested. Brackets around values indicate positive microbial growth in APT broth test.

Enzyme Activity. Viscosity measurements on pectin reaction solutions that were inoculated with laboratory test pack samples are given in Table 4. Enzymes were considered > 99.9% inactivated if the tomato inoculum caused less than 1.0% loss of viscosity in the PG assay. The limit was based on the variability in viscosity observed when using heat inactivated inocula.

At 215°F (101.7°C), enzymes were inactivated in all samples in 35 min, but not in 30 min of processing. Since it was shown earlier that acidification did not increase enzyme denaturation during heating above 80°C, apparent inactivation at some acidification levels for the 30 min process was interpreted as random occurrence associated with a marginal process; i.e., 30 min was a borderline process where some samples would be positive and others negative with respect to residual enzyme activity. At 230°F (110°C), enzyme activity was not found

TABLE 5.
VISCOSITY TESTS FOR PG ACTIVITY IN CANNERY-PACKED TOMATOES
PROCESSED AT 210°F FOR CONDITIONS IN TABLE 2. VISCOSITIES (CP) OF PECTIN
REACTION MIXTURES INOCULATED WITH COMMERCIAL TEST PACK TOMATOES
(AVG ± ONE STANDARD DEVIATION). SAMPLES WITHIN ONE ACID/YEAR
TREATMENT WERE COMPARED.

Heating Time[a] (min)	Control (no acid)	Acid in cover juice				
		Gluconic 0.55%	Gluconic 0.82%	Gluconic 1.10%	Gluconic 1.32%	Citric 0.3%
			1985 Season			
20	---	5.27±0.12*	5.27±0.03*	5.31±0.04	---	4.93±0.06*
25	---	5.07±0.17*	5.22±0.02*	5.21±0.03*	---	5.05±0.02
30	---	5.28±0.04	5.24±0.03*	5.29±0.01	---	4.91±0.03*
35	---	5.23±0.04	5.32±0.09	5.27±0.06	---	4.93±0.02*
42	5.23±0.02	5.19±0.01	5.40±0.08	5.30±0.09	---	5.03±0.03
			1986 Season			
28	3.34±0.02*	---	---	---	3.37±0.05*	3.36±0.06*
35	3.49±0.08	---	---	---	3.46±0.04	3.42±0.03
42	3.44±0.06	---	---	---	3.45±0.08	3.45±0.06

[a]Followed by 28 min cooling.
*Averages include viscosity values indicating >0.1% enzyme activity in one or more of the cans tested.

after 26 min of processing, but 20 min was not adequate. At 245°F (118.3°C), a minimum process of 25 min was necessary.

The two commercial test packs of whole peeled tomatoes processed at 210°F (98.9°C) (Table 5) behaved similarly to the laboratory test packs. In the 1985 season, enzyme inactivation was questionable with processes less than 35 min for all gluconic acid packs and processes less than 42 min for citric acid packs. In 1986, however, when fill weights and wholeness were controlled, enzyme activity occurred only in samples which were processed 28 min, regardless of the acidulant used.

Microbial Activity. Although there was no indication of swelling in any of the cans, 9 cans out of 450 processed in the laboratory were positive for viable rods. Averages that include positive responses in APT broth are bracketed in Table 4. Four of the nine positive cans were controls (no acid added), one had 0.82%, two had 1.10%, and two had 1.65% gluconic acid in the covering juice. All spoiled samples were processed 20 min or less at 230°F (110°C). No viable

organisms were found in any of the commercial samples or in the laboratory samples processed at 215°F (101.7°C) or 245°F (118.3°C).

Survival of microorganisms did not correlate with pH. However, spoilage rate was 3/11 in cans with fill weights greater than 361 g processed 20 min or less at 230°F (110°C). Similar fill weights did not cause a microbiological problem in cans processed at 245°F (118.3°C).

Sample Quality. As expected, wholeness, color and drained weights were adversely affected as process severity increased. Acidification with either citric or gluconic acid did not affect wholeness or color. Gel formation in the cover juice of tomatoes acidified with gluconic acid was not evident at any processing levels where microbes and enzymes were inactivated. By definitions of the USDA grades (1964), tomato flavor and quality were acceptable even at process levels where definite enzyme activity remained. However, since temperature response of pectic enzyme inactivation (z values between 11 and 23°C) is similar to that of some spores known to spoil acid foods (Pedersen 1929; NCA 1968; Leonard *et al*. 1975), shortening processes for canned tomatoes below enzyme inactivation levels should not be considered without further study of microbiological concerns.

CONCLUSIONS

Methods were developed to measure activities of pectin degrading enzymes in their natural tomato environment without extraction or purification. Heat resistance parameters of endogenous pectic enzymes in whole tomato homogenates were significantly different from those reported on purified enzymes. Results, in general, support commercial practices and offer an explanation for some enzyme problems in tomato concentrates.

Addition of 0.44% gluconic acid decreased heat resistance of pectin esterase and eliminated gel formation in the cover juice of canned whole peeled tomatoes. Heat resistance of polygalacturonase in tomatoes was not reduced by addition of up to 1.65% gluconic acid. Pectic enzymes in canned whole peeled tomatoes were inactivated by 35 min of processing at 98.9 and 101.7°C, by 26 min at 110°C and by 25 min at 118.3°C in a rotary pressure cooker. For high temperature processes that require high fill weights to meet drained weight requirements, control of fill weights may be critical to ensure adequate heat penetration to prevent microbial spoilage and sporadic survival of enzymes.

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DEVELOPMENT OF PRODUCTS CONTAINING MESQUITE (*PROSOPIS* spp.) POD FLOUR AND THEIR NUTRITIONAL AND ORGANOLEPTIC EVALUATION

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ABSTRACT

A 100 mesh flour was prepared from mesquite pods and its proximate chemical composition, amino acid and vitamin content determined. The flour was tested as an ingredient in beverages, pinole, candy, butter, breakfast cereals, and yogurt. Nutritional attributes of the products are discussed and ingredient cost estimates calculated. Taste panel studies indicated the most desirable mesquite flour containing products were mesquite candy, chocolate/mesquite flour beverages, and breakfast cereals with added mesquite flour.

INTRODUCTION

Pods from native mesquite (*Prosopis* spp.) trees have long held an important niche in many arid land ecosystems, often serving as one of the primary uncultivated food energy sources for indigenous man and wildlife (Felker 1979). There has recently been a resurgence of interest in the domestication and exploitation of pods from mesquite trees for use as a crop for agricultural areas faced with a diminishing water supply (Meyer *et al.* 1986). Major elements in this utilization effort are the application of modern technological methods for processing the pods into edible components (Del Valle *et al.* 1986; Del Valle *et al.* 1987) and the production of mesquite containing foods based on current consumer interests, which is the impetus for this study.

Previous papers (Del Valle *et al.* 1986; Del Valle *et al.* 1987) have described the development and evaluation of processes for manufacture of mesquite pod (*Prosopis* spp.) flour. The process that produced the optimal organoleptic quality, yield and chemical and nutritional characteristics was identified as consisting of toasting the pods, pin milling and screening the resulting flour through 45 and 100 mesh screens. The fine material through the 100 mesh screen constituted the final product. It was found that two kinds of flour could be produced by toasting either at 125°C for 40 min (lightly toasted) or 150°C for 10 min (highly toasted). These processes have been carried out commercially with good results.

Following development of the industrial process to produce mesquite flour of good quality, uses for the flours had to be defined. The purpose of this work, therefore, was to identify different applications for the mesquite flours.

MATERIALS AND METHODS

Mesquite (*Prosopis* spp.) pods were collected in the area within and adjoining Chihuahua City, Mexico, and stored in polyethylene bags in a shaded area at room temperature until used. Mesquite flour was prepared from the pods as in Del Valle *et al.* (1986, 1987) by toasting at either 125°C for 40 min or at 150°C for 10 min. The toasted pods were pin milled and sieved and the material passing through the 100 mesh sieve was used as the flour.

Proximate analysis for moisture, nitrogen, ash, fiber, reducing and nonreducing sugars and vitamins B1, B2, C, A and niacin were performed in triplicate using AOAC (1980) methods. Amino acids were determined by a modified Spackman *et al.* (1958) ion-exchange method using a Durrum model D-500 amino acid analyzer. Cysteine and methionine were determined separately as cysteic acid and methionine sulfone after performic acid oxidation. The nitrogen to protein conversion factor was calculated according to Kohler and Palter (1967). Acid detergent fiber was determined as in Goering and Van Soest (1975). The protein efficiency ratio (PER) was determined by the method of Miller (1963) and Munro and Allison (1964).

Nine products were developed utilizing the lightly toasted mesquite pod flour. All were similar to products which are currently being manufactured in Mexico and which are extensively consumed by different population groups. These were as follows.

(1) Chocolate flavored beverage. Four parts of a commercial powdered chocolate beverage base (Table 1) were mixed with one part mesquite pod flour. The beverage was prepared by blending 5 heaping teaspoonfuls of the chocolate base/ mesquite pod flour mixture in 250 mL of milk.

(2) Strawberry flavored beverage. A commercial powdered strawberry beverage base (Table 1) was mixed in a 4:1 ratio with mesquite pod flour. The beverage

TABLE 1.
INGREDIENT FORMULAS OF CHOCOLATE AND STRAWBERRY BEVERAGE
BASES AS UTILIZED FOR ENRICHMENT WITH LIGHTLY TOASTED
MESQUITE POD FLOUR

Ingredient	Strawberry Beverage Base	Chocolate Beverage Base
Sugar (sucrose)	61.10%	57.36
Soy flour (full-fat)	18.86	18.86
Powdered oats (rolled, cooked)	15.44	15.44
Tricalcium phosphate	1.75	1.75
Salt (sodium chloride)	0.40	0.55
Vitamin and mineral mix	0.11	0.11
Artificial strawberry flavor	1.80	---
Cocoa	---	4.72
Artificial chocolate flavor	---	1.00
Cinnamon flavor	---	0.18
Red coloring	0.04	---
Brown coloring	---	0.02
Cream flavor	0.50	---

was prepared by blending 5 heaping teaspoonfuls of the strawberry flavored base/ mesquite pod flour in 250 mL of milk.

(3) Sweetened "horchata" type beverage. Four grams of powdered sugar were mixed with 5 g mesquite pod flour and 0.2 g powdered cinnamon. The beverage was prepared by blending 9.2 g base in 150 mL water.

(4) Pinole. Commercial "pinole" (coarsely ground toasted corn, 40 parts) was blended with 30 parts sugar and 10 parts mesquite flour.

(5) Pressed mesquite pod flour/peanut butter candy. Commercial peanut butter (35 parts) was blended with sugar (55 parts) and mesquite pod flour (10 parts), and the resulting blend was pressed to form round cakes 4 cm in diameter × 2 cm high which were consumed without further processing.

(6) Mesquite pod butter. Five parts toasted mesquite pod flour was blended with 15 parts powdered sugar and 8 parts vegetable (safflower) oil.

(7) Farina and mesquite pod flour. Commercially produced dry Farina (Cream of Wheat) cereal (46 g), 9 g toasted mesquite pod flour and 2 g salt were boiled in 200 mL water until the usual consistency was attained.

(8) Rolled oats cooked with mesquite pod flour. Commercially produced rolled oats (94 g), 19 g mesquite pod flour and 4 g salt were boiled in 200 mL water to the desired consistency.

(9) Flavored yogurt blended with mesquite pod flour. Commercial strawberry flavored yogurt (98 parts) was blended with 2 parts mesquite flour.

Organoleptic evaluations of the products were carried out utilizing a 25-member untrained panel. Panelists scored the following quality attributes of each product utilizing a scale of 1 (minimum) to 9 (maximum): aroma, flavor, color, texture, and overall attributes.

The highly toasted mesquite pod flour was studied as an extender for cocoa or coffee. The cocoa extender application was evaluated by mixing commercially produced cocoa (Hersheys's brand) with different proportions of the highly toasted mesquite flour (0%, 25%, 50%, 75% and 100%). Five grams of the mixture were then blended with 200 mL milk and 12 g sugar. The resulting mixtures were evaluated for cocoa flavor by a five person untrained panel.

The use of highly toasted mesquite flour as a coffee extender was similarly evaluated by mixing the mesquite flour with instant coffee ("Nescafe" brand) in the same proportions (0%, 25%, 50%, 75%, 100%). Coffee/mesquite flour beverages were prepared by mixing 1.5 g of the blend in 150mL boiling water and the resulting beverages evaluated as to coffee flavor by the same five person untrained panel.

The costs of the products containing mesquite pod flour were estimated and compared with the costs of products that did not contain mesquite pod flour. The wholesale cost of the mesquite pod flour was determined by the manufacturing factory (Vega 1987); other mixture ingredient costs were based on current wholesale prices in Mexico.

RESULTS AND DISCUSSION

The proximate chemical analysis (Table 2) of mesquite pod flour is similar to results for other mesquite pod flour preparations reported earlier (Del Valle *et al.* 1987). The total sugar (28%) and the high nonreducing sugar content (17.8%) undoubtedly account for the high degree of sweetness of mesquite pod flour. An important observation from Table 2 is that the high fiber content of mesquite pod flour (26.1%) makes it an excellent fiber fortifier for foods, in addition to its natural sweetening properties.

The amino acid content of the lightly toasted mesquite pod flour (Table 3) indicates the flour is nutritionally limiting in the essential amino acids lysine, threonine, methionine + cystine, and isoleucine. This is probably due to toasting and is reflected in the relatively low PER of 1.3 reported for the flour.

TABLE 2.
PROXIMATE CHEMICAL AND VITAMIN CONTENT OF LIGHTLY TOASTED
MESQUITE POD FLOUR

Component	Percent
Moisture	3.9
Ash	4.2
Ether Extract	2.0
Protein (N x 6.07)	10.1
AOAC Fiber	16.9
Acid Detergent Fiber	26.1
Non-Reducing Sugars	17.8
Reducing Sugars	10.2

	Mesquite Pod Flour	Snap Beans Green, Raw
Vitamin B_1 (Thiamin)	<0.01 mg/100 gm	0.08 mg/100 gm
Vitamin B_2 (Riboflavin)	0.61 mg/100 gm	0.10 mg/100 gm
Niacin	3.56 mg/100 gm	0.50 mg/100 gm
Vitamin A	173 IU/100 gm	600 IU/100 gm
Vitamin C	5.49 mg/100 gm	19 mg/100 gm

The protein and fiber contents of products made with and without added mesquite pod flour are reported in Table 4. Addition of mesquite pod flour did not generally result in significant changes in protein content. A substantial increase was observed in the case of "horchata" (4.3% to 7.1%) while a substantial decrease was observed in the sweetened butters (7.8% for peanut butter vs 2.3% for mesquite butter).

In all cases, the fiber content was considerably enhanced by the addition of mesquite flour (Table 4). Increases were obtained with the chocolate and strawberry flavored beverage bases, which increased from 3.0% to 8.0%, "horchata" beverages from 4.9% to 15.2%, and cream of wheat from 3.0% to 7.0%. The commercial peanut butter contained 0.8% fiber which was increased to 5.0% fiber by the added mesquite pod flour. Smaller increases were observed in mesquite flour fortified yogurt (0.0% to 0.6%) and rolled oats (10.0% to 12.9%), although these amounts may well be nutritionally important in some situations.

TABLE 3.
ESSENTIAL AMINO ACID COMPOSITION OF LIGHTLY TOASTED MESQUITE POD
FLOUR AND FAO ESTIMATED REQUIREMENTS FOR CHILDREN

Amino Acid	Mesquite Pod Flour, Lightly Toasted g/16 g Nitrogen	FAO/WHO (1973) Children's Requirements g/16 g Nitrogen	
Isoleucine	2.7	3.7	73%
Leucine	5.8	5.6	104
Lysine[a]	3.5	7.5	47
Total Sulfur Amino Acids	2.2	3.4	65
Total Aromatic Amino Acids	5.8	3.4	170
Threonine[b]	2.6	4.4	59
Valine	4.0	4.1	98

[a] First limiting amino acid.
[b] Second limiting amino acid.

Cost estimates of the different products studied, with and without added mesquite flour, are reported in Table 4. Both the lightly and highly toasted mesquite flours would cost approximately $0.66 per kilo ($0.30 per pound). Table 4 shows that substitution with the mesquite flour would not appreciably affect the cost of most products.

The panelists' scores for the various mesquite pod flour products are shown in Table 5. The 'Overall Acceptance' panel rating was used to indicate which type of mesquite flour products would be most preferred by consumers. The products with the highest panel overall acceptance were the pressed mesquite/peanut butter candy and the chocolate flavored beverage (average scores for both were 7.1); these were closely followed by rolled oats/mesquite flour (average score 6.8) and pinole (average score 6.6). The mesquite pod butter and cream of wheat with mesquite pod flour both scored 6.5, while the strawberry flavored/mesquite pod flour beverage scored 6.4. Least acceptable were the yogurt with mesquite (average score 5.4) and the "horchata" type mesquite beverage (average score 4.4).

The texture of the beverages, candy, pinole and rolled oats were scored highest by the panel, indicating textural acceptability. The mesquite flour changed the texture of the farina product, as indicated by the lower panel score. The mesquite butter, yogurt, and horchata scored lowest.

TABLE 4.
PROTEIN AND FIBER CONTENTS AND COST ESTIMATES OF PRODUCTS WITH
AND WITHOUT ADDED MESQUITE POD FLOUR

Product	Percent Protein	Percent Fiber	Price US$
Beverage Base Without Mesquite	17.5	3.0	1.01/kg
Beverage Base With Mesquite	16.6	8.0	0.94/kg
"Horchata" (Dry Basis)	4.3	4.9	0.29/kg
Mesquite "Horchata" (Dry Basis)	7.1	15.2	0.43/kg
Pinole Without Mesquite	3.8	0.8	0.37/kg
Pinole With Mesquite	4.2	3.4	0.39/kg
Peanut Butter Candy Without Mesquite	12.0	1.2	0.11/kg
Peanut Butter Candy With Mesquite	10.3	3.7	0.10/kg
Peanut Butter, Sweetened	7.8	0.8	3.31/kg
Mesquite Pod Butter, Sweetened	2.3	5.0	1.68/kg
Farina Without Mesquite (Dry Basis)	14.0	3.0	1.15/kg
Farina With Mesquite (Dry Basis)	13.8	7.0	1.07/kg
Rolled Oats Without Mesquite (Dry Basis)	12.0	10.0	0.94/kg
Rolled Oats With Mesquite (Dry Basis)	12.2	12.9	0.90/kg
Yogurt Without Mesquite	3.5	0.0	1.11/L
Yogurt With Mesquite	3.7	0.6	1.10/L

The panel gave the highest Color rating scores of 7.0 to the chocolate/mesquite beverage, the strawberry/mesquite beverage, and the pressed mesquite/peanut butter candy followed by Pinole/mesquite flour with a score of 6.8. The lowest scores were for the "horchata" type mesquite beverage (4.7) and mesquite butter (4.4).

The highest average Flavor scores were assigned by the panel to the chocolate/mesquite beverage (7.3) and the pressed mesquite/peanut butter candy (7.0), followed by mesquite pod butter (6.6) and the cereal products cooked with mesquite pod flour (rolled oats and cream of wheat), both of which scored 6.5.

TABLE 5.
AVERAGE SENSORY PANEL SCORES[a] OF MESQUITE FLOUR PRODUCTS

Product	Overall Acceptance	Texture	Color	Flavor	Aroma
Mesquite Flour in Chocolate Beverage Base	7.1 ± 1.1	6.9 ± 0.9	7.0 ± 1.2	7.3 ± 1.2	6.7 ± 1.6
Mesquite Flour in Strawberry Beverage Base	6.4 ± 1.2	6.8 ± 1.1	7.0 ± 1.4	6.3 ± 1.2	5.3 ± 2.0
Mesquite Flour Enriched Horchata[b]	4.4 ± 1.1	4.8 ± 1.3	4.7 ± 1.4	4.8 ± 1.2	4.8 ± 1.1
Mesquite Flour Enriched Pinole	6.6 ± 1.7	6.7 ± 1.7	6.8 ± 1.7	6.3 ± 2.0	4.8 ± 2.2
Peanut Butter/ Mesquite Flour Candy	7.1 ± 0.9	6.8 ± 1.5	7.0 ±1.4	7.0 ± 1.3	5.1 ± 2.5
Mesquite Butter	6.5 ± 2.0	5.6 ± 1.8	4.4 ± 2.4	6.6 ± 2.0	3.4 ± 2.2
Farina Cooked With Mesquite Flour	6.5 ± 1.0	5.8 ± 1.5	5.9 ± 1.7	6.5 ± 1.3	5.6 ± 1.9
Rolled Oats Cooked With Mesquite Flour	6.8 ± 1.0	6.1 ± 1.6	5.8 ± 2.0	6.5 ± 1.5	5.8 ± 2.1
Plain Yogurt With Mesquite Flour	5.4 ± 1.0	5.6 ± 1.8	6.0 ± 1.8	3.6 ± 1.9	4.7 ± 2.2

[a] Results reported as mean ± standard deviation.
[b] "Horchata" is a beverage prepared from raw rice dispersed in water, which is lightly sweetened and cinnamon flavored; the product reported here was enriched with mesquite flour, as mentioned in the text.
[c] "Pinole" is a toasted corn flour, mixed with sugar; the product reported here was enriched with mesquite flour; as mentioned in the text.

Lowest scores were those of "horchata" type beverage (4.8) and the mesquite/yogurt mixture (3.6).

The highest "Aroma" scores were for the chocolate/mesquite beverage (6.7) with the lowest for the "horchata" type beverage (4.8), yogurt/mesquite mixture (4.7), and the mesquite flour butter (3.4).

Reasons given by panelists for choosing the preferred products were that mesquite pod flour conferred a malt-like flavor and a pleasant sweetness. Reasons for disliking the least-preferred products were either lack of flavor or poor consistency. It appears that mesquite pod flour does not possess sufficient flavor to be used alone, but when combined with more flavorful foods, it enhances and improves the resulting blends.

Panel tests of the highly toasted mesquite flour used as a chocolate or coffee extender demonstrated that no flavor differences could be detected in blends containing up to 50% toasted mesquite pod flour. Use of 75% mesquite flour changed the flavor of the chocolate or coffee to a pleasantly unique combination of the components. Beverages containing 100% highly toasted mesquite flour have a pleasant chocolate-like flavor similar to toasted carob, which is not surprising since carob is a close botanical relative of mesquite.

Based on the above results, possible applications of the lightly toasted mesquite flour are visualized as flavor enhancer, natural sweetener, and fiber fortifier. As a flavor enhancer, lightly toasted mesquite pod flour produces a pleasant toasted malt-like flavor which enhances the organoleptic quality of such products as beverages, pinole, peanut butter candy and hot cereals. The sweetening properties are due to the high content of sugar and could readily replace refined sugar.

As a fiber fortifier, the lightly toasted mesquite flour will double and sometimes triple the fiber content of foods without adversely affecting organoleptic quality. This form of fiber fortification has a number of advantages; the fiber is in a finely divided form which is easily incorporated into many foods, no fibrous or rough texture is apparent in the fortified products as often occurs when cereals such as wheat bran are utilized, and fortification with mesquite flour in most cases enhances the flavor of the product being fortified.

The highly toasted mesquite flour has important potential applications as a chocolate or coffee extender. Both of these applications would result in substantial savings to users of these products.

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APPLICATION OF RESPONSE SURFACE METHODOLOGY IN PROTEIN EXTRACTION STUDIES FROM BREWER'S SPENT GRAIN

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ABSTRACT

Effects of temperature of extraction, time of extraction, concentration of sodium dodecyl sulphate and Na_2HPO_4 in the extractant solution, particle size of grain and BSG:extractant ratio on the yield of protein solubilized from dried brewer's spent grain (DBSG) and pressed brewer's spent grain (PBSG) were studied simultaneously using a process optimization technique termed Response Surface Methodology (RSM). The initial fractional factorial screening design indicated that time, temperature and particle size of grain were significant variables while concentration of extractant had no effect on protein yield from either DBSG or PBSG. The mean yield of protein extracted from DBSG was 28.14% compared to 9.53% for PBSG. When a central composite rotatable design was subsequently applied to four variables, temperature, time, BSG:extractant ratio and concentration of Na_2HPO_4 in the extractant solution, with particle size of grain held constant at 1.5mm, all variables had a significant effect on protein yield from DBSG. Using multiple regression analysis, a second order polynomial model was derived and used to predict the yield of protein extracted from DBSG under various combinations of these four variables. Generation of contour plots enabled selection of levels for each variable to give a product with a desired protein concentration. Typically, a yield of approximately 60% protein could be obtained from DBSG using a concentration of 0.6% Na_2HPO_4 in the extractant solution, a BSG:extractant ratio of 2.5:100 and the mixture heated at 90°C for 95 min. The observed protein yield agreed well with the predicted maximum value using a RSM approach and demonstrated RSM as a powerful and elegant research tool when several variables are to be evaluated simultaneously.

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INTRODUCTION

Brewer's spent grain (BSG) is the principle by-product from the brewing process and is used mainly as animal feed. Recently however, there has been increasing interest in its use in food and food products such as flour mixes, bread, cookies and meat products (Prentice *et al.* 1978; Finley and Hanamoto 1980).

Several studies have been done to develop methodology for protein extraction from BSG as the relatively high protein content of BSG, along with the fact that it is a continually available biomass resource, provides a sound basis for investigating this material as a source of commercial food protein (Satterlee 1984). Vassel *et al.* (1949) reported that BSG proteins were poorly soluble in solvents commonly used for extraction of proteins, namely, water, salt, dilute acid and dilute alkali while Bhumibhamon (1981) observed that acid and alkali proteases solubilized 30% and 36% of BSG nitrogen, respectively, in 24h. Preliminary studies by Ervin *et al.* (1988) have shown that BSG protein can be readily extracted from dried brewer's spent grain (DBSG) and pressed brewer's spent grain (PBSG) using an extracting solution consisting of 3% sodium dodecyl sulphate/0.5% Na_2HPO_4 (pH7.0) followed by precipitation of proteins with ethanol in conjunction with refrigeration as shown in Fig. 1. This procedure resulted in the recovery of approximately 50% and 23% of protein as a dried powdered concentrate from DBSG and PBSG respectively (Ervin *et al.* 1988). The objectives of the present study were to determine the conditions of extraction, (shown in Fig. 1), which could be used in conjunction with each other to optimize the yield of protein concentrate from BSG. Factors under investigation included temperature of extraction, time of extraction, particle size of BSG, concentration of sodium dodecyl sulphate, and Na_2HPO_4 in the extracting solution and BSG:extractant ratio. Since several factors were to be examined simultaneously in this study, the traditional one variable at a time experimental approach used in the initial study would be approximate and time consuming and would fail to take into consideration important interaction effects. In order to overcome the limitations of the one variable at a time approach and to adequately describe the effect of several factors simultaneously, including any important interactions, a process optimization technique termed Response Surface Methodology was used. RSM enables the calculation of an optimum yield based on a few sets of experiments in which all factors are varied within chosen ranges using factorial experimental designs and multiple regression analyses (Khuri and Cornell 1987). Developed initially for process optimization studies in chemical engineering, RSM has been applied to studies of casein extrusion (van de Voort and Stanley 1984), canola sauce production (Ma and Ooraikul 1986), folacin retention in cookies (Connor and Keagy 1981) and control of mold spoilage in bakery prod-

BSG (dried or pressed)

(a) Extraction with SDS solution
(b) Heat at 100°C for 60 min
(c) Filtration to separate supernatant
    from BSG residue

Residue; 15% crude protein (%Kjeldahl N x 6.25)
(Spray dry; could be used as animal feed)

Supernatant
(a) Refrigerate (4°C)
(b) Addition of ethanol(70%) to ppt.
    protein
(c) Centrifuge (9,500 g, 0°C)

Supernatant
(Distill to recover ethanol)

Protein precipitate
(Spray dry, use as
food, animal feed)

FIG. 1. FLOW DIAGRAM OF EXTRACTION PROCEDURE OF PROTEIN FROM
BREWER'S SPENT GRAIN

ucts (Smith *et al.* 1988). This paper describes the experiments which lead to the determination of the optimal conditions required for the most efficient extraction of BSG protein and the advantages of RSM as a research tool in protein extraction studies from BSG.

MATERIALS AND METHODS

Experimental Design

In order to determine the effects of individual factors on protein extraction from dried brewer's spent grain (DBSG) and pressed brewer's spent grain (PBSG), a preliminary fractional factorial screening design was performed. The fractional factorial design chosen was a half fraction of a 2^4 factorial design, i.e., a 2^{4-1} design, as described by Box *et al.* (1978) and the factors evaluated initially included temperature of extraction (50–100°C), time of extraction (30–90 min), concentration of sodium dodecyl sulphate, (1–3%) and particle size of grain (1–2mm). The levels of each factor were selected from previous experimental results (Ervin *et al.* 1988) and practical possibilities for optimization of protein yield from BSG. In the screening design, the concentration of Na_2HPO_4

TABLE 1.
A HALF FRACTION OF A 2^4 FACTORIAL DESIGN (CODED) TO DETERMINE
FACTORS INFLUENCING EXTRACTION OF PROTEIN FROM DRIED BREWER'S
SPENT GRAIN (DBSG) AND PRESSED BREWER'S SPENT GRAIN (PBSG)

| Run#[*] | Variable[**] | | | | Protein recovered (%) | |
	X_1	X_2	X_3	X_4	DBSG	PBSG
1	-1	-1	-1	-1	15.81	5.04
2	1	-1	-1	1	36.28	9.86
3	-1	1	-1	1	18.85	7.06
4	1	1	-1	-1	45.99	15.68
5	-1	-1	1	1	14.85	5.37
6	1	-1	1	-1	35.61	11.43
7	-1	1	1	-1	22.28	8.07
8	1	1	1	1	36.09	14.11

 * Each run replicated twice for a total of 16 runs
 ** X_1 = Temperature ^0C; X_2 = Time (min); X_3 = Conc. of extractant (SDS); X_4 = Particle size of grain.

in the extractant solution was kept constant at 0.5%v/v and the BSG:extractant ratio (w/v) was maintained at 5:100 throughout the design. The fractional factorial coded design for both DBSG and PBSG is shown in Table 1 and all experimental runs were carried out in a randomized order. Values of coded levels used in the fractional factorial design and method of coding, as described by Box *et al.* (1978), are shown in Table 2. A more elaborate 4 factor, 5 level Central Composite Rotatable Design (CCRD) of Box *et al.* (1978) was used for fitting second order response surfaces. Factors and levels of each factor in the CCRD (Table 5) were selected on the basis of significant regression coefficients generated from the initial screening design as those likely to optimize the response. In the CCRD, particle size was kept constant at 1.5mm and the meal:solvent ratio (w/v) was varied from 2.5:100 to 12.5:100. The concentration of sodium dodecyl sulphate (SDS) in the optimization design was kept constant at 0.5% and the concentration of Na_2HPO_4 in the extracting solution was varied from 0–1%v/v. The coded

TABLE 2.
VARIABLE LEVELS AND CODED VALUES USED IN A HALF FRACTION FACTO-
RIAL SCREENING DESIGN FOR PROTEIN EXTRACTION FROM DRIED BREWER'S
SPENT GRAIN (DBSG) AND PRESSED BREWER'S SPENT GRAIN (PBSG)

	Coded Levels*		
Variable	-1	0	+1
Temperature, ^{0}C. (X_1)	50	75	100
Time, min, (X_2)	30	60	90
Concentration of SDS,** % (X_3)	1	2	3
Particle size*** of grain, mm (X_4)	1	1.5	2

* Coded variable $(-1, 1)$ = Actual value -0.5 (High value + Low value)

0.5 (High value − Low value)

** SDS = Sodium dodecyl sulphate containing 0.5% Na_2HPO_4 (pH7.0)
*** BSG:extractant ratio = 5:100(w/v)

levels of -2, -1, 0, $+1$, $+2$ used in a four factor CCRD (Table 5) were obtained from Box et al. (1978) and values of coded levels of variables used in the CCRD are shown in Table 6.

Procedure

Commercially produced DBSG (8.4% moisture, 4.51% Kjeldahl N, dry weight basis) and PBSG (74.4% moisture, 5.06% Kjeldahl N, dry weight basis) were obtained from Molson Breweries of Canada Ltd., Montreal, Quebec. Repre-sentative samples (500 g) of DBSG were ground (Mikro Sample Mill, Pulverising Co., Summit, NJ) to pass through 1–2mm sieves to give the required particle size of grain shown in the factorial screening design and the CCRD. For PBSG, previously frozen samples were thawed at room temperature, homogenized in a Waring blender and treated as described for DBSG.

For extraction of proteins in the preliminary fractional factorial screening design, five-gram samples of DBSG and PBSG were mixed with 100 mL amounts of extractant containing 1–3% SDS and 0.5% Na_2HPO_4 (pH 7.0) and the mixtures heated at the time-temperature combinations specified in the experimental design (Table 1). In the CCRD, five-gram samples of DBSG were refluxed with 100 mL of 0.5% SDS solution containing 0–1% v/v Na_2HPO_4 for the required time-temperature combination shown in the CCRD (Table 5). The BSG was separated from the supernatant by filtration using a California Buchner funnel and no vacuum. Proteins were precipitated by the addition of 95% ethanol followed by refrigeration at 4°C as described by Ervin *et al.* (1988). The precipitated proteins were recovered by centrifugation (9500 g, 0°C), washed with ethanol and ly-ophilized. Nitrogen contents of the protein concentrates were determined using a micro-Kjeldahl method (A.O.A.C. 1980; 47.021). The factor of 6.25 was used to convert % N to % crude protein.

Statistical Analyses

Statistical analyses (regression coefficients, analysis of variance and correla-tion coefficients) were computed using the Statistical Analysis System (SAS 1982). All 3 dimensional graphs and 2 dimensional contour plots were done using the SAS/Graph program on a McGill University mainframe and a Zeta Plotter.

RESULTS AND DISCUSSION

Response Surface Methodology is a process optimization technique which determines optimum conditions by combining special experimental designs with modeling by first and second order polynomial equations in a sequential testing procedure. Initially in this study, a 2^{4-1} fractional factorial screening design was carried out (Table 1) and the actual levels of variables used in each experimental run are shown in Table 2. The main advantage of this class of design is that it enables one to study three or more variables simultaneously in a single experi-mental design of practicable size by confounding the main factors in their higher order interactions and to determine which variables have an important effect on the chosen response (Mullen and Ennis 1985).

The regression equations obtained from the initial screening design for both DBSG and PBSG are shown in Eq. 1 and 2, respectively.

$$Y = 28.14 + 10.24X_1 + 2.55X_2 - 1.04X_3 - 1.73X_4 \qquad [1]$$

$$Y = 9.52 + 3.13X_1 + 1.72X_2 + 0.23X_3 - 0.54X_4 \qquad [2]$$

TABLE 3.
ANALYSIS OF VARIANCE OF PRELIMINARY FACTORIAL SCREENING DESIGN FOR
PROTEIN RECOVERED FROM DRIED BREWER'S SPENT GRAIN (DBSG)

Source	dF[*]	Sum of Squares	Mean Square	F
Due to regression	4	1848.26	462.01	88.20[a]
Temperature (X_1)	1		1678.54	320.39[a]
Time (X_2)	1		104.44	19.94[b]
Conc. of SDS (X_3)	1		17.38	3.32[ns]
Particle size of grain (X_4)	1		47.88	9.14[c]
Residual (Error	11	57.63	5.24	
Total	15	1905.89		
R^{2}[+]	0.97			

* Degrees of freedom
Level of significance, $a = p < 0.001$
Level of significance, $b = p < 0.01$
Level of significance, $c = p < 0.1$
ns = non significant
+ Coefficient of determination

The analysis of variance (ANOVA) for the preliminary screening designs are shown in Tables 3 and 4, respectively. The ANOVA for Y, yield of protein recovered, indicated that both models were highly significant (p <0.001) and had R^2 values of 0.97 and 0.96, respectively, i.e., accounting for 97% and 96% of experimental variation after being corrected for the mean. Examination of the fitted coefficients for both equations using the F-test (1 d.f.) showed that temperature of extraction (X_1), time of extraction (X_2), and particle size of grain (X_4) were significant variables and could be used to optimize protein yield while concentration of extractant had no effect on protein yield from DBSG or PBSG.

TABLE 4.
ANALYSIS OF VARIANCE OF PRELIMINARY FACTORIAL SCREENING DESIGN FOR
PROTEIN RECOVERED FROM PRESSED BREWER'S SPENT GRAIN (PBSG)

Source	dF^*	Sum of Squares	Mean Square	F
Due to regression	4	209.25	52.31	73.43[a]
Temperature (X_1)	1		156.37	219.51[a]
Time (X_2)	1		47.26	66.35[a]
Conc. of SDS (X_3)	1		0.87	1.23[ns]
Particle size of grain (X_4)	1		4.73	6.64[c]
Residual (Error	11	7.84	0.71	
Total	15	217.08		
R^{2+}	0.96			

* Degrees of freedom
Level of significance, $a = p < 0.001$
Level of significance, $c = p < 0.1$
ns = non significant
+ Coefficient of determination

The effect of temperature on protein recovery from BSG confirmed previous results which showed an increase in nitrogen solubility from BSG as the temperature of extraction increased (Ervin *et al.* 1988). The lack of effect of SDS concentration was surprising since it was assumed that higher concentrations of SDS would result in greater solubilization of BSG proteins and hence an increased yield of recovered protein. The average yield of protein extracted from DBSG was approximately three times higher than the amount extracted from PBSG (28.14% compared to only 9.53%) which agreed with previous results of Ervin *et al.* (1988). In all likelihood, this difference is related to the lower dry matter content of PBSG.

TABLE 5.
CODED LEVEL COMBINATIONS FOR A FOUR VARIABLE CENTRAL COMPOSITE
ROTATABLE DESIGN TO OPTIMIZE YIELD OF PROTEIN EXTRACTED FROM DRIED
BREWER'S SPENT GRAIN (DBSG)

Run#*	Variable**				Protein recovered from DBSG (%)	
	X_1	X_2	X_3	X_4	Predicted	Observed
1	-1	-1	-1	-1	42.39	45.97
2	1	-1	-1	-1	45.19	47.36
3	-1	1	-1	-1	44.21	46.09
4	1	1	-1	-1	46.28	47.96
5	-1	-1	1	-1	47.74	49.84
6	1	-1	1	-1	50.48	50.36
7	-1	1	1	-1	51.87	53.39
8	1	1	1	-1	51.28	49.73
9	-1	-1	-1	1	24.93	25.43
10	1	-1	-1	1	28.39	27.57
11	-1	1	-1	1	27.84	29.82
12	1	1	-1	1	32.45	31.91
13	-1	-1	1	1	32.79	33.40
14	1	-1	1	1	42.54	43.28
15	-1	1	1	1	38.07	39.60
16	1	1	1	1	36.13	39.69
17	-2	0	0	0	38.86	37.76
18	2	0	0	0	48.29	51.28
19	0	-2	0	0	37.43	36.29
20	0	2	0	0	50.34	52.86
21	0	0	-2	0	27.91	25.90
22	0	0	2	0	44.34	45.78
23	0	0	0	-2	57.41	57.71
24	0	0	0	2	27.03	26.81
25	0	0	0	0	51.46	53.05

* Each run replicated twice for a total of 50 runs
** X_1 = Temperature, (°C); X_2 = Time, (min); X_3 = Conc. of phosphate, (%); X_4 = BSG:extractant ratio

Response Surface Methodology was then applied to predict the optimal levels of temperature and time of extraction, level of Na_2HPO_4 in the extractant solution and BSG:extractant ratio in combination with one another which could be used to optimize the yield of protein extracted from DBSG. Although particle size was significant in the screening design, it was kept constant in the response surface design at 1.5mm. Since the concentration of SDS had no effect on protein yield, its concentration was kept constant at 0.5% in the CCRD and the concentration of Na_2HPO_4 in the extractant solution varied from 0–1%. The Central Composite Rotatable Design (5 levels for 4 variables) used for fitting the second order response surface and uncoded levels used in each experimental run are shown in Tables 5 and 6, respectively. CCRDs have $2^k + 2k + 1$ treatment combinations where k equals the number of variables under study and the experimental design is said to be rotatable since the variance of the predicted response, Y, at designated points, (X's), is a function only of the distances of the points from the centre, rather than a function of their direction. This implies that the variance contours of Y are concentric circles and a design with this property will leave the variance of Y unchanged when the design is rotated about the center (0,0,0,0), leading to the term "rotatable" (Khuri and Cornell 1987).

Table 5 shows the protein recovered with the corresponding coded values of temperature of extraction (X_1), time of extraction (X_2), concentration of Na_2HPO_4

TABLE 6.
VARIABLE LEVELS AND CODED VALUES USED IN CENTRAL COMPOSITE ROTATABLE DESIGN FOR PROTEIN EXTRACTION FROM DRIED BREWER'S SPENT GRAIN (DBSG)

Variables	Levels				
	-2	-1	0	1	2
Temperature, ^0C (X_1)	80	85	90	95	100
Time, min, (X_2)	60	75	90	105	120
Conc. of phosphate,% in SDS* (X_3)	0	0.25	0.5	0.75	1.0
BSG:extractant ratio % w/v (X_4)	2.5:100	5:100	7.5:100	10:100	12.5:100

* Concentration of Na_2HPO_4 in 0.5% sodium dodecyl sulphate

in the extractant (X_3) and BSG:extractant ratio (X_4). The second order polynomial model using multiple regression analysis of the uncoded data is presented as Eq. 3:

$$Y = -463.89 + 10.03X_1 + 1.22X_2 + 26.61X_3 - 208.77X_4$$
$$- 0.06X^2_1 - 0.01X^2_2 - 49.38X^2_3 - 619.85X^2_4 + 0.04 X_1X_2$$
$$+ 0.36 X_1X_3 + 2.53X_1X_4 + 0.03X_2X_3 - 0.09X_2X_4 + 28.36X_3X_4 \qquad [3]$$

Examination of the ANOVA of the fitted model (Table 7) indicated that the F-value and overall correlation coefficient (r) of the model were significant at $p < 0.05$, the equation having an $R^2 = 0.87$ accounting for 87% of the variation in terms of extracted protein. The $F_{0.05}$ value for lack of fit (0.53) did not exceed the tabulated value of 2.57 (9,26 d.f.) indicating that lack of fit was not significant and therefore the fitted model was appropriate for the description of the response surface. With the regression coefficents obtained, the stationary point of the fitted surface was computed using the equations suggested by Khuri and Cornell (1987). The predicted yield, together with the coded and uncoded levels of variables at the stationary point, X_0, are shown in Table 8.

TABLE 7.
ANALYSIS OF VARIANCE FOR SECOND ORDER POLYNOMIAL MODEL FITTED TO
YIELD OF PROTEIN (%) EXTRACTED FROM DRIED BREWER'S SPENT GRAIN
(DBSG)

Source	dF	Sum of Squares	Mean Square	F
Due to regression	14	4214.71	301.05	16.65
Residual (Error)	35	633.06	18.08	
Lack of Fit*	9	93.81	10.42	0.503^{ns}
Pure Error	26	539.25	20.74	

* Lack of Fit Sum of Squares (SS) = Residual SS - Pure Error SS
ns = non significant

TABLE 8.
CODED AND UNCODED VALUES OF VARIABLES AT STATIONARY POINT X_0
(POINT OF MAXIMUM YIELD OF EXTRACTED PROTEIN)

Variable	Coded	Uncoded
Temperature, ^0C (X_1)	0.2	91
Time, min, (X_2)	0.53	98
Conc of phosphate,% in SDS (X_3)	0.56	0.64
BSG:extractant ratio % w/v (X_4)	-2	2.5:100

Predicted yield of extracted protein at stationary point = 58.95%w/w
Observed yield of extracted protein at stationary point = 58.45%w/w

To determine the nature of the stationary point, canonical analysis, as described by Khuri and Cornell (1987), was carried out on the second order polynomial model to transform the fitted model to a new coordinate system with the origin at X_0. The axes of the system were then rotated until they were parallel to the principal axis of the response surface. The canonical form of the equation demonstrating the nature of the response surface was:

$$y = 58.95 - 0.008\,w_1^2 - 0.05w_2^2 - 49.03\,w_3^2 - 619.41w_4^2$$

where w_1, w_2, w_3 and w_4 are the axes of the response surface. It is evident that all the eigenvalues, i.e. -0.008, -0.05, -49.03 and -619.41 were all negative indicating that the stationary point was a maximum with the surface slightly extended towards the w_1 axis (Khuri and Cornell 1987).

The linear, quadratic and cross-product terms in the second order polynomial were used to generate 3 dimensional response surface graphs (Fig. 2–3) and 2
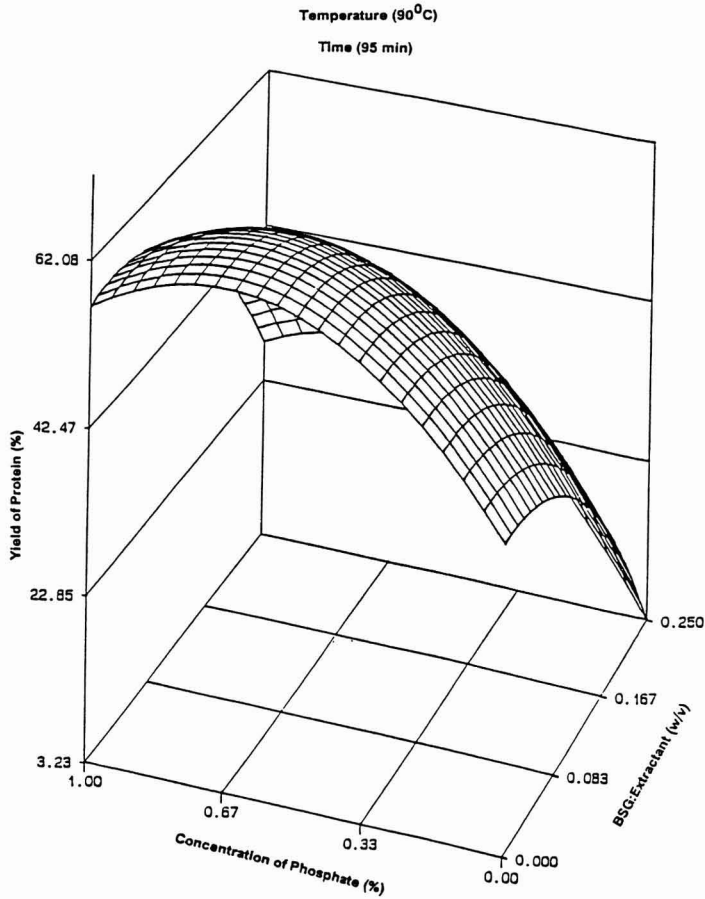
Figure 2 (R. Diptee et al. 1989)

FIG. 2. THREE DIMENSIONAL RESPONSE SURFACE GRAPH SHOWING THE EFFECT OF PHOSPHATE CONCENTRATION IN SDS SOLUTION AND MEAL:SOLVENT RATIO, WITH TEMPERATURE AND TIME OF EXTRACTION HELD CONSTANT AT 90°C AND 95 MIN, RESPECTIVELY, ON PROTEIN YIELD (%w/w) FROM DBSG

dimensional contour plots (Fig. 4–5) of protein yield from DBSG. As Fig. 2 illustrates, an increase in the Na_2HPO_4 concentration in the extracting solution and a decrease in BSG:extractant ratio with temperature and time of extraction held constant, resulted in an increase in protein yield from DBSG. The protein yield could also be increased by increasing temperature and time of extraction while keeping the concentration of Na_2HPO_4 and BSG:extractant ratio constant, as shown in Fig. 3. While these graphs can assist the researcher to determine the direction to take to increase a desired response and graphically show the
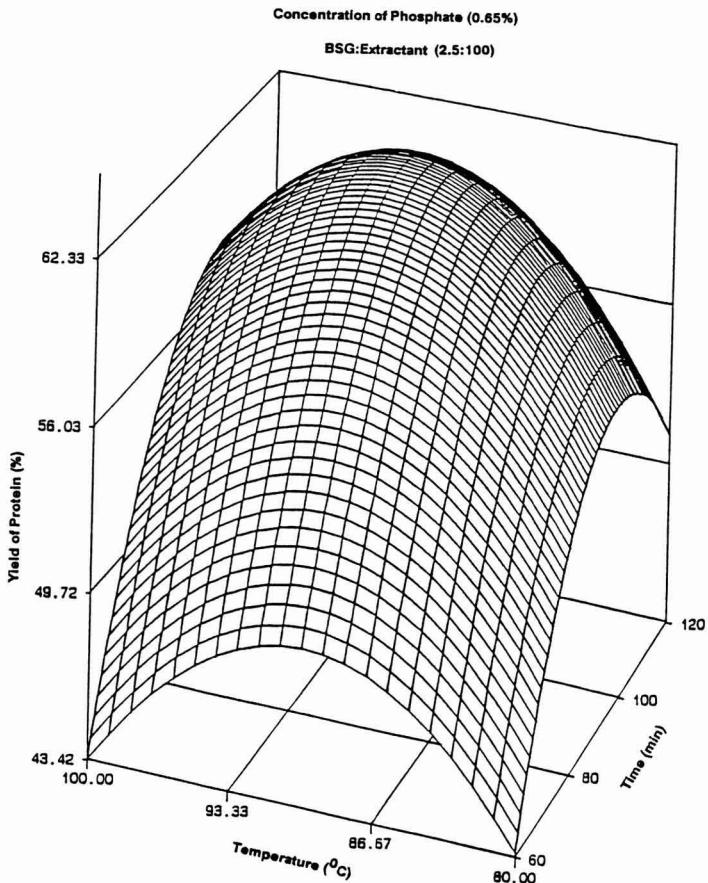
Figure 3 (R. Diptee et al. 1989)

FIG. 3. THREE DIMENSIONAL RESPONSE SURFACE GRAPH SHOWING THE EFFECT
STORAGE TEMPERATURE AND TIME OF EXTRACTION, WITH CONCENTRATION OF
PHOSPHATE IN SDS SOLUTION AND MEAL:SOLVENT RATIO HELD CONSTANT AT
0.65% AND 2.5:100, RESPECTIVELY, ON PROTEIN YIELD (%w/w) FROM DBSG

nature of the fitted surface as maximum, minimum or a saddle point, it is difficult
to determine levels of variables to give a specific protein yield from graphs such
as these. This can more readily be achieved from contour plots of the same
variables, examples of which are shown in Fig. 4 and 5, respectively. By referring
to these plots, levels of factors can be selected to predict protein yield extracted
from DBSG. For example, a protein yield of 40% w/w could be obtained by
extracting DBSG in 0.5% SDS/0.1% Na_2HPO_4 using a BSG:extractant ratio of

Temperature (90°C)

Time (95min)
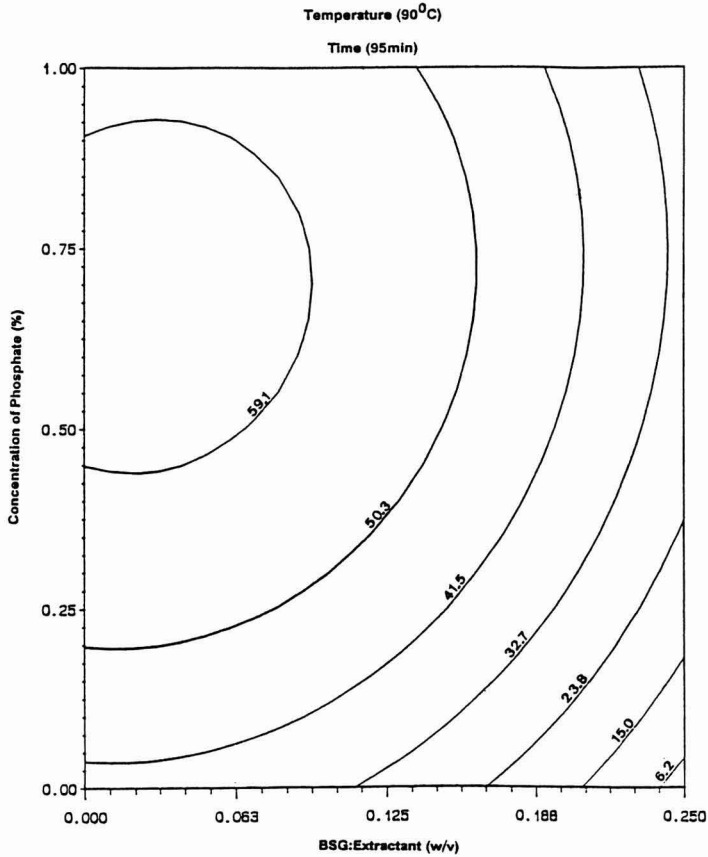
FIG. 4. TWO DIMENSIONAL CONTOUR PLOT SHOWING THE EFFECT OF PHOSPHATE
CONCENTRATION IN SDS SOLUTION AND MEAL:SOLVENT RATIO, WITH TEMPERA-
TURE AND TIME OF EXTRACTION HELD CONSTANT AT 90°C AND 95 MIN, RESPEC-
TIVELY, ON PROTEIN YIELD (%w/w) FROM DBSG

14:100 and heating the mixture at 90°C for 95 min (Fig. 4). A higher protein
yield (55% w/w) could be obtained at the same time-temperature combination
by decreasing the BSG:extractant ratio to 4:100 and increasing the Na_2HPO_4
concentration in the extractant solution to 0.9% v/v (Fig. 4). A yield of 55% of
extracted protein could also be obtained by decreasing the concentration of
Na_2HPO_4 to 0.65% in the extracting solution and the BSG:extractant ratio to
2.5:100 and heating the mixture at 95°C for 80 min (Fig. 5). Using the conditions

Concentration of Phosphate (0.65%)

BSG:Extractant (2.5:100)
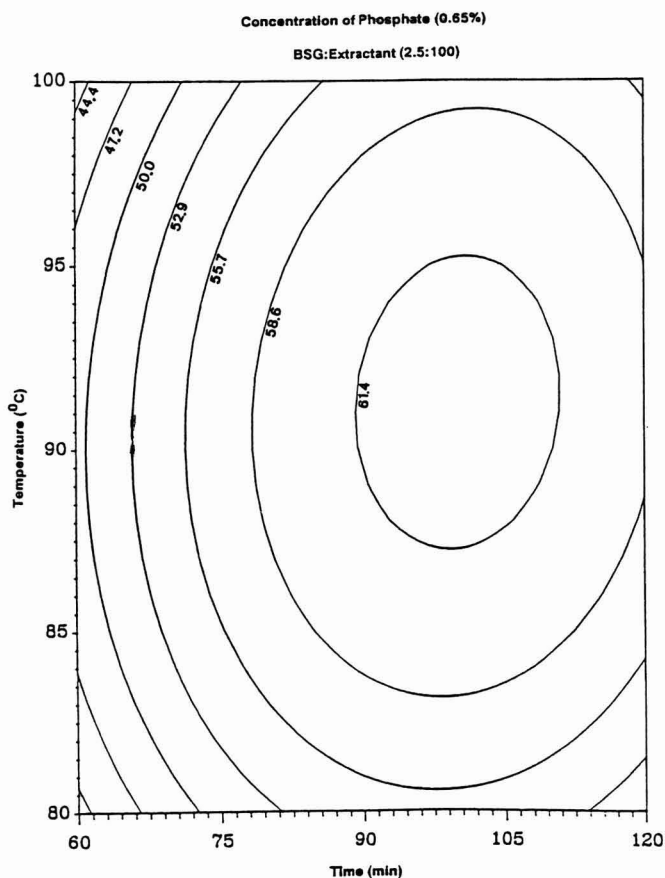
Figure 5 (R. Diptee et al. 1989)

FIG. 5. TWO DIMENSIONAL CONTOUR PLOT SHOWING THE EFFECT STORAGE TEM-
PERATURE AND TIME OF EXTRACTION, WITH CONCENTRATION OF PHOSPHATE IN
SDS SOLUTION AND MEAL:SOLVENT RATIO HELD CONSTANT AT 0.65% AND
2.5:100, RESPECTIVELY, ON PROTEIN YIELD (%w/w) FROM DBSG

obtained at the stationary point, i.e., 91°C, 98 min, 0.64% Na_2HPO_4/0.5% SDS
and BSG:extractant ratio of 2.5:100, a protein yield of 58.45% (mean of 10
replicates) was obtained which closely agreed with the calculated yield of 58.9%
(Table 8).

In conclusion, RSM is an elegant statistical technique that uses quantitative
data to determine and simultaneously solve multivariate equations which specify
the optimum protein yield for a specified set of factors through mathematical

models. This study has shown that by using RSM the scientist and/or manufacturer can consider a much wider range of combinations and factors to predict protein yield from DBSG than would normally be possible using the one variable at a time approach. According to Henika (1982) the advantages of RSM in food research can be summarized as follows:

(1) it provides more information in less time at less cost than the one variable at a time approach;
(2) it suggests combinations of the most important variables, processing and storage conditions to give a desired response and;
(3) it allows the presentation of results in a readily understood graphical form.

While RSM has many advantages, it does have certain limitations, the major one being the selection of factor levels under investigation which are generally based on literature values, previous screening designs, practical experience, on regulatory constraints or on commercial specifications. Secondly, the results obtained by regression analyses are only estimates of protein yield from DBSG for a particular set of data and may vary for another set of experimental data. Thirdly, while the regression data can be used to predict the amount of protein extracted, it is inadvisable to extrapolate beyond the range of levels of factors actually used in experimental runs.

Despite these minor limitations, RSM is a powerful and elegant research tool when several variables are to be evaluated simultaneously. It is hoped that this application assists in making food researchers more aware of the RSM technique and the many advantages it offers in comparison to the traditional approach to experimentation in protein extraction studies from plant materials.

ACKNOWLEDGMENTS

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KINETICS OF METHYLMETHIONINE SULFONIUM IN BUFFER SOLUTIONS FOR ESTIMATING THERMAL TREATMENT OF LIQUID FOODS[1]

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ABSTRACT

Reaction kinetics of MMS degradation in sodium citrate buffer solutions followed a first order reaction kinetics in the temperature range of 121.1°C–132.2°C and the pH range of 4 to 6. Reaction rate constant ranged from 0.1260 to 1.1003 min⁻¹ and the corresponding D-values varied from 18.28 min to 2.09 min depending on the pH and temperature. Activation energy (117.1 - 135.4 kJ/mole) or Z-values (22.8 - 20.0°C) were slightly dependent on pH. The kinetic data was used for estimating potential decimal reduction in B. Stearothermophilus *spores.*

INTRODUCTION

The thermal processing of foods is one of the most prevalent methods used to preserve both low and high acid foods. Destruction of pathogenic and degradative microorganisms and enzymes is the primary objective of this process. However, other food quality characteristics such as organoleptic, cosmetic and nutritive elements may also be affected. All of these factors can be quantitatively measured or indexed against the time and temperature program to which they are subjected, i.e., the degree of thermal treatment of a product can be tied to a measurable index. Typically this index has been the thermal destruction of microorganisms to indicate the relative safety of the food product. Microbiological assays are often time consuming, completed several days after the process, subject to recovery and contamination problems, and require large population changes as evidence of the process. An alternative approach would be to use a

measurable change in thermosensitive molecules as an index of the product's thermal treatment. The potential for rapid and timely analysis, elimination of recovery and contamination problems, and opportunity to measure at nanomolar or less levels suggest that some sort of chemical index may be more useful than using microorganisms in quantifying the extent of thermal treatment a product has received. In addition, chemical compounds may be used which are indigenous to the food, allowing for the continuous monitoring of a thermal process and being more natural in reflecting the actual heat treatment than would the use of indicator organisms which may be influenced by or may influence the heating characteristics of the product.

Using microbiological inoculated packs as indicators for the thermal processing of foods is a routine technique in the food industry. Utilization of chemical indices or other similar techniques has received little attention. Bounie and Cheftel (1986) demonstrated the use of peroxidase enzymes as indicators to characterize heating during extrusion; the change in meat texture was used by Tennigen and Olstad (1979) as a heating index; and Pettipher (1986) used anthocyanins as an indicator for the extent of fermentation cocoa leaves. Mulley *et al.* (1975) demonstrated the use of thiamine as a chemical index for sterilization efficacy and suggested that this approach is a "fertile ground for research and patent hunters"; this prediction has not yet developed probably due to the lack of identified compounds and their kinetics in various food systems. Lund (1975) summarized information on reaction rate versus temperature relationships for many microorganisms, enzymes, nutrients and quality factors in food. Okos (1986) edited a book which further expands this data base.

Destruction of microorganisms, textural changes, organoleptic change, nutrient destruction, color changes, development of new compounds and other physical-chemical changes in foods as a result of thermal processing have been typically found or classified as first order reactions (Lund 1975; Feliciotti and Esselen 1957; Mulley *et al.* 1975; Toledo 1986; Holdsworth 1985; Stumbo 1973). The slope of line obtained by plotting log concentration versus time is termed as the reaction coefficient (k), and can through a series of equations be used to develop the kinetics for compounds or microorganisms of interest.

Toledo (1986) suggested that since chemical degradation and microbiological inactivation of thermally processed foods are both first order reactions, they could be coupled in an expression to demonstrate their relationship in changes that occurs during processing. This assumes that the z-value is constant over the range of temperatures evaluated and that temperature is constant for any given process. Caudron (1988) rederived Toledo's equation to correct assumptions made regarding the Arrhenius equation. The correlation between microbial inactivation and chemical degradation is expressed as:

$$\log \frac{N_o}{N} = [\frac{D_{Mo}}{D_{Co}} 10^{(T_o - T)} (\frac{1}{z_m} - \frac{1}{z_c})]^{-1} \log \frac{C_o}{C} \quad (1)$$

where N and N_0 are viable microorganisms at time t and 0, respectively; C and C_0 represent the concentration of undegraded chemical components at time t and 0, respectively; T_0 and T are the reference temperature and process temperature, respectively; Z_m and Z_c are the Z-values in the temperature range of 121.1°C to 132.2°C for microbial and chemical species, respectively; and D_{Mo} and D_{Co} are the D-values at the reference temperature for the microbial and chemical species, respectively. Log N_0/N expresses the number of decimal reductions for the microorganisms during the process. Development of this equation is found in Berry (1988).

Methylmethionine Sulfonium is an appropriate prototype for investigation as an indicator compound in thermal processing. It is widely found in plants, with over 58 fruits and vegetables containing measurable levels. Its chemical reaction due to heat has been characterized and it is recognized to break down stoichio-metrically to homoserine and dimethyl sulfide which can also be quantified. The development of kinetic data covering the high to low acid food range in a model system is an initial step in utilizing MMS as an indicator compound.

The objectives of this project were: (1) to provide the kinetic data from the thermal degradation of MMS with which the D-values and Z-values for MMS can be estimated, and (2) to use Eq. (1) for correlating the destruction of MMS with a low acid food sterility indicator microorganism.

MATERIALS AND METHODS

Experimental Design

A Latin Squares statistical design (Table 1) was employed to eliminate any sample or process bias (Neter and Wasserman 1974). Several experiments were conducted prior to the design of the Latin Square to evaluate time factors as influenced by temperature and pH. Since there is no published information on the effect of other environmental factors, such as oxygen concentration, light, ionic strength, trace metals, etc., on degradation kinetics of methyl methhionine sulfonium ion, we excluded these factors from our experiments.

Chemicals and Solutions

DL-methionine-S-methyl sulfonium chloride (MMS), dl-homoserine (HOM), dl-norleucine (NOR), and dl-pipecolic acid (PIP) representing two different lots, each were obtained from Sigma Chemical Co. (PO Box 14508, St. Louis, MO); likewise two different lots, each of citric acid and dibasic sodium phosphate were purchased from Mallinckrodt Inc. (St. Louis, MO) for preparation of buffer solutions. The lots were randomly divided into two groups, such that solution one (mix 1) would only have chemicals designated as group 1 and solution two (mix 2) would only have chemicals from group 2. Solutions of MMS (100 mM),

TABLE 1.
LATIN SQUARES EXPERIMENTAL DESIGN FOR MMS THERMAL PROCESSING AT
VARIOUS LEVELS OF TEMPERATURE AND pH

Temperature (°C)	pH 4 5 6 Time (min.)					
121.1	0	1	6	10	14	18
126.7	0	1	3	6	12	16
132.2	0	1	2	3	6	9

Processed in 6 TDT retorts at 6 positions within each retort.

HOM (100 mM), NOR (50 mM) and PIP (50 mM), were prepared using deion-ized distilled sterile filtered water for use as internal standards. NOR, PIP, and MMS solutions were combined at a 1:1:1 volume ratio and then added to pH 4, pH 5 or pH 6 buffer solutions at 3 : 97 ratio by volume. This resulted in a 1 mM solution of MMS with 0.5 mM internal standards of PIP and NOR. The buffer solutions were prepared according to McIlvaine (1921) and tested for pH using a Corning pH meter (Model 125). All solutions were stored at 4°C prior to use, and used within 24 h.

Thermal Processing

Ten mL solutions were dispensed into 208 × 006 TDT cans (American National Can Company, Barrington, IL), which had previously been cleaned with petroleum ether and auto-claved for 20 min. Cans with lids were seamed on an Automatic Master Sealer (Wisconsin Aluminum Foundry Co., Inc., Man-itowoc, WI). Seal integrity was evaluated by pre- and post-process weighing of the cans; no seal problems (i.e., change in weight) were encountered. All filled TDT cans were immediately frozen at -40°C until they were processed. Prior to testing, product was thawed for one hour at room temperature ($\approx$20°C), the TDT cans were then placed in a 7 slot rack, 6 cans with product according to the experimental design plus one can in the middle slot with a type ''T'' thermocouple placed in it to record the temperature. The thermocouple was inserted in the base of the can using a stuffing box for a retortable pouch (Item C-5.2, O. F. Ecklund, Cape Coral, FL). A 24 gauge thermocouple wire was used; the wire entered the TDT retort through the same type of stuffing box installed in the pod's lid. A cube of styrofoam was used to keep the wire tip from touching the can surface, therefore only recording the liquid temperature. A continuous strip chart recorder

(Digistrip II, Kaye Instruments, Bedford, MA) was used to record the time and temperature. The rack of cans was placed in the TDT retort. The heating process occurred with constant steam venting followed at process completion with a cold water chill. Once processing was completed, the cans were refrozen at -40°C until the contents were assayed.

Analytical Preparation — Ion Exchange and Amino Acid Derivatization

Thawed cans were punctured, the solutions withdrawn by pipette and stored refrigerated in glass vials until used. The procedure for ion exchange and amino acid derivatization is detailed in Berry (1988) and Berry et al. (1989). Minor modifications were made to improve the volume consistency in the ion exchange columns by increasing the aliquot of ion exchange resin from 1.4 to 1.6 mL. Also the evaporator was changed to a Multihead Evaporator (Model III, Organ-omation Assoc., Inc.).

Statistical Analysis

SAS Institute (1985) general linear model was used to develop the statistical data base; the Bonferroni test and Dunn's multiple range test were used to compare the means.

RESULTS AND DISCUSSION

Response Factors

Values for calculating MMS were determined based on the zero time response of MMS to the internal standards, NOR and PIP (Table 2). NOR was selected as the internal standard for most of the calculations.

TABLE 2.
MMS RESPONSE FACTORS AT VARIOUS pH'S RELATIVE TO NOR AND PIP. USED FOR CORRELATING MMS LOSS; MEASURED IN SODIUM CITRATE BUFFER SOLUTIONS

		NOR			PIP			
Solution	Value	N	S.D.[a]	C.V.[b]	Value	N	S.D.	C.V.
Dd Water	.4798	6	.0363	7.566	.5147	6	.0327	6.351
pH 4	.4753	19	.0590	12.409	.4987	19	.0604	12.118
pH 5	.4804	26	.0706	14.691	.5040	26	.0744	14.766
pH 6	.4670	19	.0805	17.238	.4829	22	.0819	16.971

[a] S.D. = standard deviation.
[b] C.V. = coefficient of variation.

Statistical Analysis

To reduce experimental error, a Latin Squares experimental design was employed. To expand the base of the data, the two different mixes of solutions, were statistically analyzed and had no significant differences at $P>.05$ (Table 3). Consequently, the mix data was pooled for the analysis of the design using the General Linear Model.

The components of the design were temperature (treated separately), placement (order) of TDT cans in the retort pods, retort time, pH, day of process, and interactions between day of placement, time and pH (Table 4). As expected, significant differences $(P>.05)$ were found within retort times and within the pH of the various solutions in their effects on MMS degradation for all three processing temperatures (121.1, 126.7, 132.2 °C). Again as expected (and desired), the day of processing and interactions between can placement and pH were not significant $(P>.05)$ for the three temperatures. What was unexpected was a significant interaction between processing time and day for 121.1°C which should not have occurred; no such interactions were observed for the 126.7 and 132.2°C process. The interaction between day and time at 121.1°C suggested that the same processing times had significantly different effects on MMS destruction on different days. A closer examination of the results showed that samples processed in TDT retort number 4 out of total 6 had analytical values for MMS that were outliers compared to the other values. When the outliers were removed from the statistical analysis, it was found that time and day no longer had significant interactions. The analysis of the experimental design showed only significant effects for time and pH with temperature and therefore the kinetics for MMS could be developed without influence of other interactions.

TABLE 3.
COMPARISON OF THERMALLY PROCESSED MIX 1 AND MIX 2 MMS CHEMICAL
LOTS. MIXES PREPARED IN SODIUM CITRATE BUFFER SOLUTIONS, AND
PROCESSED IN THE TDT RETORTS AT INDICATED TEMPERATURES. NOR USED
AS INTERNAL STANDARD.

| | PR > F | | |
| | Process Temperature | | |
pH	121.1°C	126.7°C	132.2°C
4	.9549[a]	.5735[a]	.4500[a]
5	.6514	.5869	.7041
6	.6652	.2314	.7732

[a] The means within each lot were not significantly different according to the Bonferroni T Test at $P>.05$.

TABLE 4.
GENERAL LINEAR MODEL STATISTICAL ANALYSIS OF THE LATIN SQUARES
MODEL DESIGN COMPONENTS FOR THE CHEMICAL KINETICS OF MMS. NOR AS
INTERNAL STANDARD. $P > .05$.

Design Component	Degrees of Freedom	PR > F Process Temperature 121.1°C	126.7°C	132.2°C	Comment
Placement In Retort Pod	5	.2648	.3531	.2102	N.S.[b]
Retort Time	4	.0001	.0001	.0001	Significant effect
Treatment (pH)	5	.0003	.0051	.0002	Significant effect
Process Day	1	.2889	.0637	.1357	N.S.
Day * Placement	5	.8169	.1188	.6882	N.S.
Day * Time	4	.0117[a]	.6657	.9927	N.S.
Day * Treatment (pH)	5	.5157	.9295	.9999	N.S.

[a] When outliers from pod 4 are removed $PR>F$ becomes .2732 and is not a significant effect.
[b] N.S. = not significant.

The outlier data points were not eliminated from the data base used to develop the kinetic information; this was in part due to the inability to establish a cause for the outliers such as analytical error or equipment malfunction (Neter and Wasserman 1974).

Chemical Kinetics of MMS

Table 5 summarizes the kinetic equations used in determining k, D-value, Z-value and activation energy for MMS. Figure 1 shows the heat penetration curve for the TDT thermal process; the cans reached process temperature within 15 s which suggests that the system was well mixed. This time lag should not affect reaction rate, k, according to Feliciotti and Esselen (1957). They suggested that if the thermal destruction of the compound (MMS) followed a first order reaction rate, then the initial concentration does not have influence on the characteristics of the reaction. Therefore the small losses encountered during the heating and cooling periods do not affect the nature of the rate constants. The loss of MMS was evaluated for both pH and temperature over time and plotted as classical least squares lines for first order chemical reactions as shown in Fig. 2.

TABLE 5.
REACTION KINETIC EQUATIONS FOR MMS AND MICROORGANISM DESTRUC-
TION. USED IN CALCULATING THE D-VALUE, HALF-LIFE ($t\frac{1}{2}$), z-VALUE AND
ACTIVATION ENERGY (Ea) FOR A FIRST ORDER REACTION (LUND 1975).

DESCRIPTION	SYMBOL	EQUATION	UNITS
1. First Order Reaction	k	$\log \dfrac{C}{C_o} = \dfrac{-k}{2.303} t$	min^{-1}
2. D-value (Log Base)	D	$D = \dfrac{2.303}{k}$	min
3. Half-life	$t\frac{1}{2}$	$t\frac{1}{2} = \dfrac{0.693}{k}$	min
4. z-value	z	$\log \left(\dfrac{k_1}{k}\right) = \left(\dfrac{T_o - T}{z}\right)$	°C
5. Activation Energy	E_a	$E_a = \dfrac{2.303 \ RTT_o}{z}$	J/g mole

Where: C_o = initial conc. of reactant
C   = conc. of reactant at any time
t   = time
T   = reference temperature, K
T   = temperature at Z°C less than T_o in K
R   = gas constant 8.314 kJ/kg K

The slopes of Fig. 2 was used to obtain the reaction velocity coefficients, k, for the first order reactions. These are shown in Table 6, along with the D-value, and half life for the destruction of MMS. As the pH increased from 4 to 6, the reaction rate constant increased by 2.54 times at 121.1°C and 2.84 times at 132.2°C. When temperature increased from 121.1°C to 132.2°C, the reaction rates for pH 4 increased 3.07 times while at pH 6 they increased 3.6 times. Williams (1973) found similar trends when measuring DMS, a product of MMS thermal degradation, over the range of 82°C to 99°C in citrate buffers at pH's of 4, 5, 6 using gas chromatography. Kovatscheva (1978), using thin layer chromatrography found similar values over a temperature range of 62°C to 122°C in citrate buffers from pH 4 to pH 8. The reaction rate increases as the pH goes from 4 to 6 confirming that the reaction is strongly base driven. This had been suggested by Ramirez et al. (1973).

Table 7 shows the Z-values for pH of 4, 5, 6. The values are somewhat similar but slightly pH dependent. The increasing value for activation energy from pH 4 to pH 6 is consistent because the MMS degradation reaction is base driven. Williams (1973) found similar values for Z-value and activation energy (E_a).

TDT TEMPERATURE PROFILE
10ML SOLN MODEL SYSTEM - 6 MIN. PROCESS
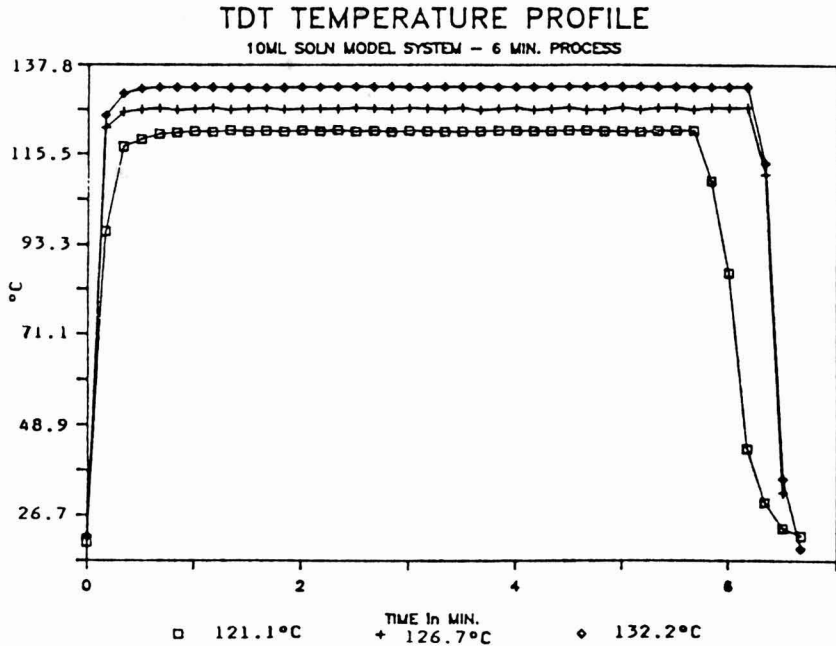
FIG. 1. HEAT PENETRATION CURVE FOR THERMAL PROCESSING OF MODEL
SOLUTIONS IN TDT CANS
"T" type thermocouple placed in center can of a 7 can rack and processed in a TDT retort.
Legend: °C: 121.1 (□), 126.7 (+), 132.2 (◇).

MMS Concentration and Microbe Reduction

To compare the decimal reduction of a standard microorganism often used as a sterilization indicator to the change in concentration of MMS due to thermal processing, *Bacillus stearothermophilus* (BS) was selected. Literature values (Stumbo 1973) for BS used were D_{Mo} = 4 min at pH 5 and 5 min at pH 6, Z_m = 7.8°C at pH 5 and 12.2°C at pH 6 and T_o = 121.1°C. These values were used for the evaluation of decimal reduction in BS due to decimal reduction in MMS using Eq. (1). The relationship between decimal reduction in MMS concentration and decimal reduction of BS for pH 5 and 6 at varying temperatures is shown in Fig. 3. It appears that MMS is best suited for indexing the microbial lethality at high temperature and low pH combination as compared to that of low temperature and high pH combination.

It should be noted that Z-value of MMS degradation is quite high (20°C) as compared to Z-value of BS inactivation. Therefore, in a system which is not well mixed and where temperature gradients occur (e.g., canned foods), care

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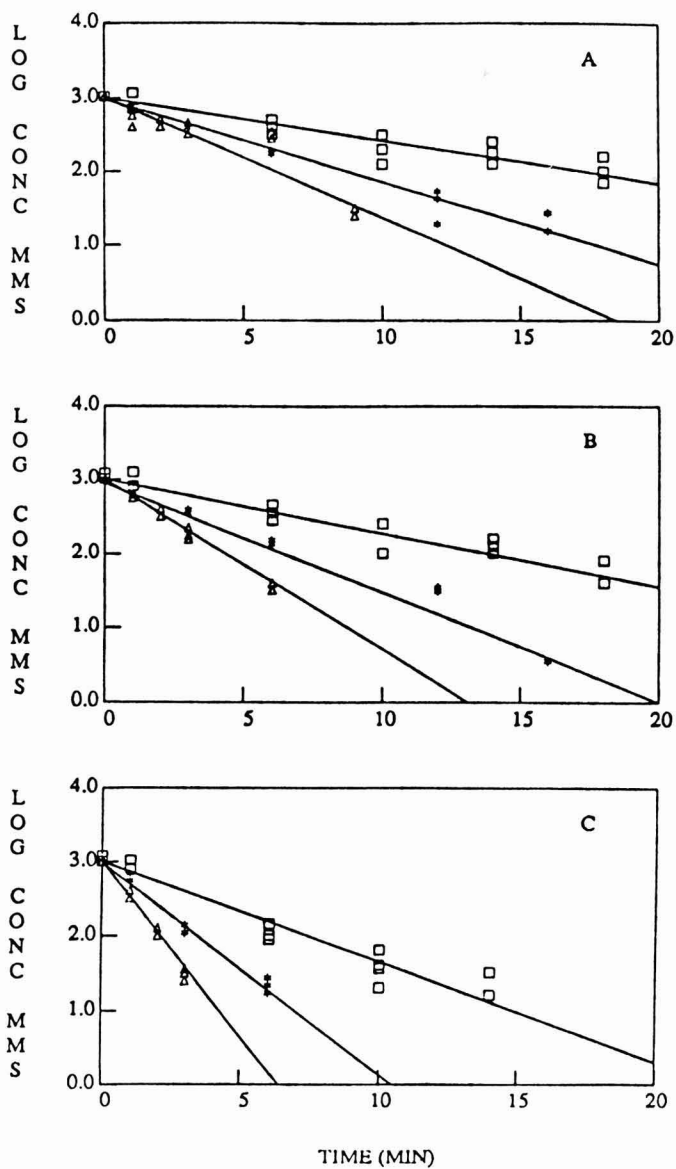
FIG. 2. FIRST ORDER REACTION KINETIC CURVES FOR MMS DEGRADATION A-pH 4, B-pH 5, c-pH 6; 121.1°C (□), 126.7°C (*), 132.2°C (Δ).

TABLE 6.
REACTION VELOCITY COEFFICIENTS (k), D-VALUES AND HALF-LIFE (t½) FOR
MMS DESTRUCTION. DETERMINED IN SODIUM CITRATE BUFFER SOLUTIONS
WITH TEMPERATURE BETWEEN 121.1 AND 132.2°C BASED ON NORLEUCINE AS
THE INTERNAL STANDARD. THE COEFFICIENT OF CORRELATION (R^2) IS
REPORTED FOR THE REGRESSION EQUATION USED FOR GENERATING k.

Temperature (°C)	pH	$k(min^{-1})$	$D(min)$[a]	$t_{\frac{1}{2}}(min)$	R^2
121.1	4	0.1260	18.28	5.5	.9048
	5	0.1559	14.77	4.4	.9089
	6	0.3058	7.53	2.3	.8333
126.7	4	0.2572	8.95	2.7	.9521
	5	0.3351	6.87	2.1	.9573
	6	0.6937	3.32	1.0	.9766
132.2	4	0.3876	5.95	1.8	.9693
	5	0.5375	4.28	1.3	.9641
	6	1.1003	2.09	0.6	.9752

[a]D = time required to reduce the concentration of a component by 90%.

must be taken such that the residual MMS in all parts of the product be deter-
mined. By contrast, the low Z-value of microorganisms usually result in neg-
ligible number of survivors around the portions of the product which is the fastest
heating, therefore, survivors at the slowest heating part of the material usually
represent most of the survivors.

TABLE 7.
Z-VALUE AND ACTIVATION ENERGY (E_a) FOR MMS DESTRUCTION. DETERMINED
IN SODIUM CITRATE BUFFERED SOLUTIONS WITH TEMPERATURES BETWEEN
121.1 AND 132.2°C BASED ON NORLEUCINE AS THE INTERNAL STANDARD.

pH	Z °C[a]	E_a $(\frac{kJ}{mole})$
4	22.8	117.1
5	20.7	130.4
6	20.0	135.4

[a] Temperature change required to reduce D by 90%.
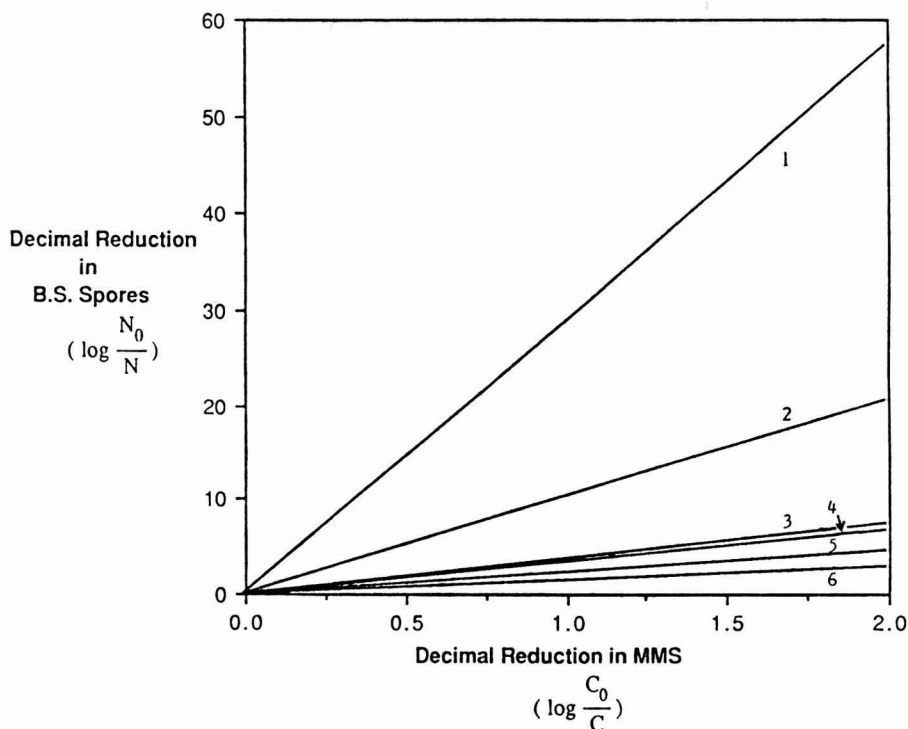
FIG 3. DECIMAL REDUCTION IN *B. STEAROTHERMOPHILUS* SPORES AS A FUNCTION
OF DECIMAL REDUCTION IN MMS CONCENTRATION 1:132.2°C & pH 5, 2:126.7 &
pH 5, 3:121.1°C & pH 5, 4:132.2°C & pH 6, 5:126.7°C & pH 6, 6:121.1°C & pH 6.

CONCLUSION

Kinetics of MMS show it to be a first order reaction over a pH range of 4 to 6, covering the area from high and low acid foods. Kinetic data was developed for the UHT aseptic processing range of 121.1°C to 132.2°C. Reaction rate constants were both pH and temperature dependent. The rate constants increased 2.54 times or more as the pH became more basic going from pH 4 to 6, while rate constants increased more than 3 times when the temperature was increased by 11.1°C. The kinetic data was used to correlate MMS destruction with reduction of microorganisms *B. stearothermophilus*.

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