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D.B. LUND
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ANNOUNCEMENT

Preliminary Information and Invitation
International Conference on Food Physics
May 25-27, 1994
University of Horticulture and Food Industry, Budapest, Hungary

The International Society of Food Physicists (ISFP) and the Editorial Board of Journal of Food Physics cordially invite you for participation in the Conference to be held at the University of Horticulture and Food Industry.

Registration fee: $150.00
Accommodation: in student hostel and hotels
Working groups:

(1) Rheology of foodstuffs, rheological measurement technique, rheological parameters
(2) Radioactivity of the foodstuffs, radiation methods in the agrofood sector
(3) Nondestructive physical methods (e.g. NIR, NMR. INAA) for investigation of foodstuffs
(4) Physical methods (e.g., heating, irradiation) for treatment of foodstuffs during food processing
(5) Basic questions (theoretical background, history, etc.) of food physics
(6) Technical development, instrumentization, measurement technique, automatization, control of food industry.

We plan some review-type and short (maximum 15 min) lectures. The lecturers should send short abstracts (maximum 1 page). The material of the Conference will be published as a special issue of Journal of Food Physics.

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Arrival: May 24, 1994 or_____
Departure: May 28, 1994 or_____
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CONTENTS

THE EFFECTS OF SOLUTION COMPOSITION AND DRYING TEMPERATURE ON CRYSTALLINITY, PERMEABILITY AND MECHANICAL PROPERTIES OF METHYLCELLULOSE FILMS

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ABSTRACT

The effects of film drying temperature (100C for 35 min, 80C for 1 h, 50C for 1.5 h or room temperature overnight) and ethanol concentration (0, 25, 50 or 75%) in the aqueous film solution on the physical properties of methylcellulose (MC) films were investigated. Increased drying temperatures increased crystallinity in all films. MC films prepared from a 75% water-25% ethanol solvent exhibited smaller permeabilities to oxygen and water vapor, greater crystallinity (when the higher drying temperatures were used), greater tensile strength, and greater percent elongation than films prepared from other water or water/ethanol solvents. Ethanol, at a 25% concentration, may enhance intermolecular hydrogen bonding of MC, while higher concentrations of ethanol may have prevented complete hydration of MC. Extensive association of MC by hydrogen bonding and hydrophobic association (which induces crystallization) likely reinforces the film matrix and presumably produces a film with greater resistance to oxygen and water vapor.

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INTRODUCTION

Methylcellulose (MC) is a cellulose ether formed by alkali treatment of cellulose, followed by reaction with methylchloride (Grover 1982). The maximum possible substitution of methoxyl groups on the anhydroglucose molecule is three [degree of substitution (DS) is 3.0]. MC used in food must have an average DS of 1.64–1.92 (Grover 1982).

MC exhibits thermal gelation and it also has excellent film forming properties. MC has been used extensively in both the food and pharmaceutical industries (Anon. 1982; Ononokpono and Spring 1987, 1988; Reading and Spring 1984; Rowe 1982; Wan and Prasad 1987). However, the use of MC films in the food industry is not common. Recently, MC films have been combined with lipids to make edible films that can serve as effective barriers to moisture migration between food components of differing a_w (Greener and Fennema 1989a, b; Hagenmaier and Shaw 1990; Kamper and Fennema 1985; Kester and Fennema 1989; Rico-Peña and Torres 1990). MC films have also been tested as barriers to lipid migration in confectionery products (Nelson and Fennema 1991).

Although MC films are usually prepared from aqueous solutions, MC is also soluble in aqueous ethanol (Anon. 1982). Several investigators have incorporated ethanol in MC film solutions to increase viscosity, to solubilize film additives, and to decrease drying time, but the effects of ethanol on film properties have received little attention (Greener and Fennema 1989a, b; Kamper and Fennema 1985; Kester and Fennema 1989; Rico-Peña and Torres 1990; Vojdani and Torres 1990). The addition of 20% ethanol to a waterbased MC solution also increases its gelation temperature by approximately 20C (Anon. 1982).

Although published reports on the effect of solvent type on physical properties of MC films were not found, Tamba Vemba and Roland (1980) reported that the tensile strength, water vapor permeability, and oxygen permeability of films of ethylcellulose (EC) were dependent on the solvent used in the film solution. Since MC and EC have similar chemical compositions, it is likely that the physical properties of MC films would also be dependent on the solvent used in the film solution.

Procedures for making MC films vary not only in the solvents used, but also in the drying times and temperatures (Nelson and Fennema 1991; Ononokpono and Spring 1987; Reading and Spring 1984; Wan and Prasad 1987). The final moisture content achieved during drying is important since Ononokpono and Spring (1987) found that this influenced the ease with which the film could be removed from a glass support. Nelson and Fennema (1991) experienced difficulty in removing dried MC films from glass plates, especially when the films were less than 0.013 mm (0.5 mil) thick.

The objectives of this study were to examine the effects of drying temperature and ethanol concentration on the crystallinity, oxygen and water vapor permeabilities, tensile strength, and elongation properties of MC films.

MATERIALS AND METHODS

Methylcellulose Film Solutions

The compositions of MC film solutions are listed in Table 1. Solutions in which water was the only solvent were prepared by dispersing all of the MC (A15 LV; Dow Chemicals, Midland, MI) in one-half the water (twice distilled, 90C) with continuous agitation, adding the remaining water (10C), and cooling to 10–15C. Film solutions were stored overnight at 5C to help assure complete hydration and to free the solution of visible air bubbles.

Other MC film solutions were prepared similarly, except MC was first dispersed in warm ethanol (EtOH; 100%, 60C), followed by addition of the water (5–10C). Solutions were then cooled with agitation, and stored as described above.

TABLE 1.
COMPOSITION OF METHYLCELLULOSE FILM SOLUTIONS[a]

Solvent Composition (v/v)	Methyl-cellulose (g)[b]	Water (ml)[c]	Ethanol (ml)[d]
100% H_2O	6.0	200.0	0.0
75% H_2O-25% EtOH	6.0	150.0	50.0
50% H_2O-50% EtOH	6.0	100.0	100.0
25% H_2O-75% EtOH	6.0	50.0	150.0

[a] Dried films were 0.025 ± 0.005 mm (1.0 ± 0.2 mil) thick.
[b] Methylcellulose (A15 LV; Dow Chemicals, Midland, MI).
[c] Water (twice distilled).
[d] Ethanol (100%)

Film Formation

MC films were prepared by pouring 35 ml of film solution in a confined area of 306 cm^2. Confinement was achieved with a plastic frame. Films were dried in a forced-air oven at 100C for 35 min, 80C for 1 h, 50C for 1.5 h, or at room temperature overnight (23–25C, ambient air and relative humidity [RH]). Temperatures were chosen to encompass the incipient MC gelation temperatures of 54C and 75C, respectively, for 100% H_2O-MC and 75% H_2O-25% ETOH-MC solutions (Grover 1982). The drying times were chosen to produce films that were free from obvious traces of solvent.

Films were removed from glass plates after drying. Some films dried at 50, 80 and 100C could not be easily removed from the glass plates even when they were allowed to stand overnight at ambient conditions. Dried films were approximately 0.025 $\pm$ 0.005 mm (1.0 $\pm$ 0.2 mil) thick. Prior to testing for permeability, crystallinity or mechanical properties, films were equilibrated (1 week) in an atmosphere at 52% RH achieved using a saturated solution of magnesium nitrate. This RH was chosen because it is realistic in practice, and higher RHs caused excessive hydration of the film and inconsistent values for water vapor permeability (WVP).

Film used for x-ray diffraction (XRD) tests were prepared by pipetting 1 ml of film solution on a g cm^2 area of glass. Samples were dried using the four conditions mentioned above. After drying and prior to testing, samples were equilibrated at 52% RH.

Testing Procedures

X-Ray Diffraction. Copper-K_α radiation (λ = 1.5418 Å) was generated using a Scintag/USA PAD-V Diffractometer (Scintag, Inc., Santa Clara, CA). Samples were examined using an accelerating voltage of 45 Kv and a current of 40 ma. A diffracted angle range of 3–30 °2Θ was scanned for initial identification of diffraction peaks, while a range of 6–10 °2Θ was used to quantify differences in the degree of crystallinity among films. Films were scanned at a rate of 1 °2Θ/min. Three replicates of each film type were examined.

Oxygen Permeability. Oxygen permeability (O_2P) of MC films was determined at 25C using an Ox-tran 100 (Modern Controls, Inc., Minneapolis, MN). The carrier gas was 1% H_2/99% N_2 and the test gas was oxygen. Both the carrier gas and oxygen were conditioned at 52% RH by passage over aqueous glycerol (G) (ASTM 1971). A stainless steel mask was used to decrease the exposed film area to 5 cm^2. Unless otherwise noted, at least four replicates of each MC film were tested.

Water Vapor Permeability. Water vapor (WV) transmission rate through films was determined using a Permatran W1-A instrument (Modern Controls, Inc., Minneapolis, MN). Samples were tested using a 52–0% RH gradient and

an airflow of 15 ml/min across the dry side of the film. A saturated solution of magnesium nitrate was placed in the test cell to produce 52% RH (Rockland 1960) on one side of the film. The Permatran was calibrated using a 0.023 mm (0.92 mil) polyester film supplied by Modern Controls, Inc. Stainless steel masks were used to decrease the transmission area to 5 cm^2. WVP were calculated from the transmission values (Donhowe and Fennema 1993).

Film Thickness. A micrometer was used to measure film thickness. Reported thickness values are means of five measurements.

Mechanical Properties of MC Films. The mechanical properties of MC films were tested using an Instron Universal Testing Machine (Model 100, Instron Co., Canton, MA) and a modified ASTM method (ASTM 1988). MC films were cut into 12.7 × 1.3 cm strips, and film thickness and width were accurately measured at four places along the midsection of the film strip. Prior to testing, strips were stored at room temperature and 52% RH for 7 days. Film samples were secured with pneumatic rubber grips, leaving 7.6 cm of film between the grips. A strain rate of 0.2 mm/s was used. At least five replicates of each film were tested.

Tensile strength and elongation properties were determined from the resulting stress-strain curve. Tensile strength was calculated by dividing the maximum load on the film before failure by the initial cross sectional area. Percent elongation was calculated by measuring maximum extension of the film between the initial grip separation (7.6 cm), dividing this value by 7.6, and multiplying by 100.

Statistical Analysis

Data were initially evaluated using analysis of variance (ANOVA; SAS® Statistical Package; SAS Institute, 1985). When the ANOVA test indicated a significant ($\alpha = 0.05$) difference among means, a least significant difference test was used to identify which film means differed significantly.

RESULTS AND DISCUSSION

Qualitative Observations

MC Solutions. MC solutions containing distilled water, 75% H_2O-25% EtOH, or 50% H_2O-50% EtOH as a solvent were clear. The MC solution containing 25% H_2O-75% EtOH was turbid, apparently because of undissolved particles of MC.

MC Films. All MC films dried at room temperature (23–25C), regardless of solution composition, could be separated from the glass plate without undue

stress to the film. However, all MC films dried at 80C or 100C, adhered tenaciously to the glass plate and could be removed only with the aid of a razor blade. When dried at 50C, films made using water as the solvent could be separated from the glass plate fairly easily, but those films made using water-ethanol blends as a solvent adhered strongly to the glass plate. Because films dried at temperatures above 50C often could not be easily removed from the glass plates, only films dried at room temperature were used for measuring permeability and mechanical properties.

MC solutions containing water as the only solvent or a blend of 75% H_2O-25% EtOH became opaque during the intermediate stages of drying at 80C and 100C (became clear at the termination of drying). This opaqueness is indicative of MC gel formation (Heymann 1935). However, solutions containing the 50% H_2O-50% EtOH and 25% H_2O-75% EtOH solvent blends did not form gels and did not become opaque during the intermediate stages of drying. These results agree with Heymann's (1935) observation that MC solutions containing more than 30% EtOH will not undergo a sol-gel transformation.

Dried films made using 100% H_2O or 75% H_2O-25% EtOH as solvents were clear. However, MC films made using 50% H_2O-50% EtOH as a solvent had a speckled appearance, caused apparently by precipitated particles of MC. Films made using 25% H_2O-75% EtOH as a solvent exhibited much larger regions (2–3 cm in diameter) of presumably undissolved MC, separated by thin lamellae of clear film. This solution, as mentioned, was turbid before drying.

X-Ray Diffraction (XRD) Properties

XRD patterns for MC films made using water as a solvent and dried at either room temperature or 80C are shown in Fig. 1. The MC films dried at room temperature displayed two diffraction peaks, a sharp peak at 8.25 °2Θ and a broad peak at 20.5–21.5 °2Θ. The sharp peak corresponds to a d spacing of 10.71 Å, and this represents the d_{101} reflection in the cellulose ether crystalline lattice (Vasil'ev *et al.* 1973). Kato *et al.* (1978) and Mark and Susich (1930) attributed this peak to the trimethylglucose repeating unit. This peak will shift to larger spacings with increasing DS on the glucose molecule (Vasil'ev *et al.* 1973).

The broad peak at 20.5–21.5 °2Θ, corresponding to a d spacing of 4.13–4.33 Å, represents the $d_{\bar{1}01}$ reflection in the crystalline lattice. This reflection is independent of DS on the glucose molecule (Vasil'ev *et al.* 1973).

Both spacings are in excellent agreement with values reported in the literature (Kato *et al.* 1978; Mark and Susich 1930; Vasil'ev *et al.* 1973). The d_{101} spacing varied only $\pm$ 0.1 Å with different drying conditions and ethanol concentrations.

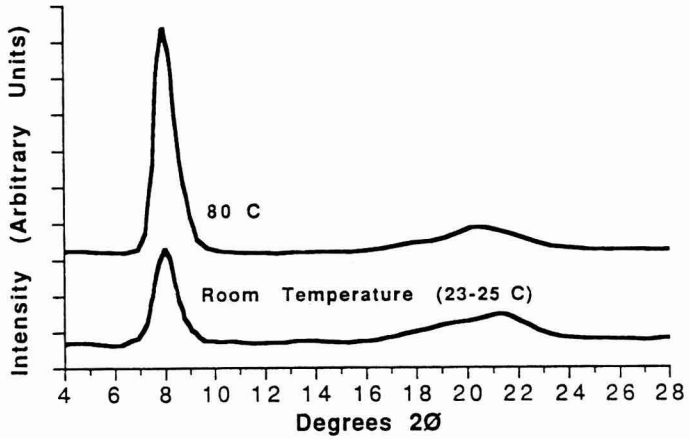

FIG. 1. X-RAY DIFFRACTION PATTERN OF METHYLCELLULOSE FILMS DRIED AT ROOM TEMPERATURE (23-25C) or 80C

Curves have been separated to provide greater clarity. Baselines of initial curves were superimposed.

The intensity of the diffraction peak at 20.5–21.5 °2Θ was constant, irrespective of drying conditions and ethanol concentration. However, the diffraction peak at 8.25 °2Θ increased in intensity with increasing drying temperature (Fig. 2), and was dependent on the solvent used to prepare the films. Use of the 75% H_2O-25% EtOH solvent generally resulted in the greatest diffraction intensity, particularly when drying temperatures of 80C or 100C were used. The peak intensities of films prepared using some EtOH were greater than those obtained when water was the only solvent, except for the film made using 25% H_2O-75% EtOH as a solvent, which produced the lowest peak intensity among all the films when dried at temperatures above 50C.

By comparing the intensities of the d_{101} diffraction peaks (8.25 °2Θ) in MC films, a rationale for the dependence of MC crystallization on drying temperature and solvent composition of MC solutions can be developed. This rationale involves consideration of solubility of MC in the solvent and ability of MC to form a gel.

Effect of Drying Temperature. The effect of drying temperature on crystallization of MC films is influenced by whether or not gel formation precedes drying. The presence of methoxyl groups on the anhydroglucose molecule is responsible for the unique phenomenon of reversible, thermal gelation displayed by MC solutions. Several theories have been advanced to explain heat-induced aggregation of MC molecules. Heymann (1935) reported that MC thermal gelation resulted from dehydration of MC molecules at elevated temperatures. Rees (1969) proposed two theories for MC thermal gelation, one

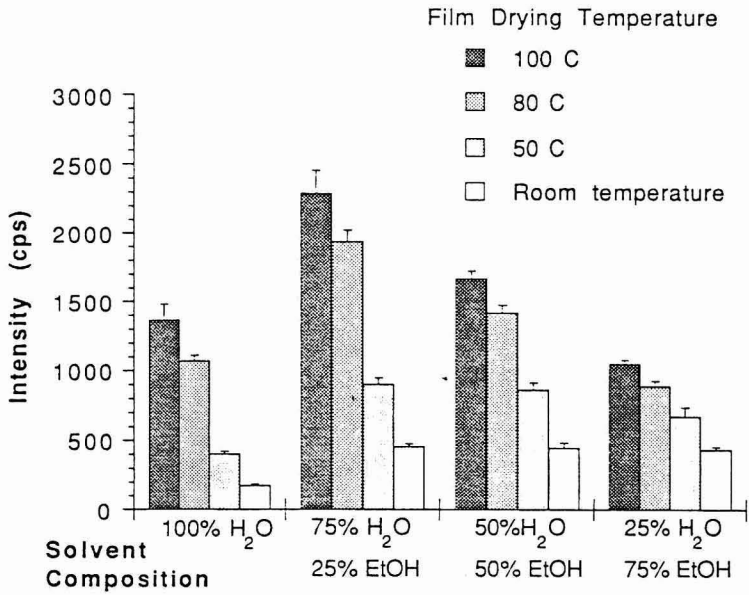

FIG. 2. EFFECT OF SOLVENT COMPOSITION AND DRYING TEMPERATURE ON INTENSITY OF THE X-RAY DIFFRACTION PEAK AT 8.25 °2θ (REPRESENTS d_{101} SPACING OF 10.71 Å) IN METHYLCELLULOSE FILMS
Intensities (in counts per second) are means of three trials. Error bars represent standard deviations.

involving destabilization of hydrogen bonds between the ether oxygen groups and water at high temperatures, and one suggesting micelle formation. Kato *et al.* (1978) supported the latter theory of Rees (1969) and reported that densely substituted regions of MC formed droplets, or micelles, when the solution is heated. This phenomenon was attributed to disruption of water structure near hydrophobic groups of MC. It was proposed that the resulting micelles act as "cross linking loci" for gel formation. Further investigation by Kato *et al.* (1978) revealed that the "cross linking loci" of MC gels consisted of crytalline trimethylglucose sequences.

It is likely that gel formation during drying would contribute to a higher degree of crystallinity in films, because MC gels possess some crystalline order (Kato *et al.* 1978). Gel formation begins at approximately 54C in the 100% H_2O-MC solution and at about 75C in the 75% H_2O-25% EtOH-MC solution (Grover 1982). Solutions consisting of 50% H_2O-50% EtOH-MC and 25% H_2O-75% EtOH-MC did not gel during heating and all solutions were sols at room temperature.

If gelation and ensuing crystallization of MC are hydrophobic processes, as suggested by Kato *et al.* (1978), then drying at elevated temperatures would likely enhance crystallization, since hydrophobic interactions are favored at high temperatures. The crystalline intensities shown in Fig. 2 indicate that

crystallinity does indeed increase with increasing drying temperature for each type of film, and that solutions that form gels, those with solvent systems of 100% H_2O and 75% H_2O-25% EtOH, possess substantial crystallinity.

Effect of Ethanol Concentration. At the two lowest drying temperatures (50C and room temperature), films prepared from solvents containing ethanol always exhibited greater crystalline intensities than those of films prepared using pure water as a solvent (Fig. 2). At low drying temperatures, all solutions were sols because the lowest gelation temperature among these solutions is 54C (Grover, 1982). Thus, at low temperatures, crystallization would be primarily instigated by dehydration of MC molecules rather than by gel formation, which occurs at higher temperatures.

The presence of EtOH in MC solutions would be expected to facilitate crystallization by encouraging EtOH-H_2O interactions, lessening H_20-MC interactions and thereby enhancing MC-MC interactions. This was found to be true (Fig. 2), except when MC solutions containing 75% ETOH were dried at either 80 or 100C. The most likely explanations for this aberrant result are the decreased solubility of MC with increasing concentrations of EtOH and the possible interference of abundant ETOH with gel formation. These same explanations can be used to explain the declining ability of ETOH to enhance film crystallinity as ETOH concentration in the solvent phase of the film solution is increased from 25 to 75% (Fig. 2).

The high degree of crystalline order in films prepared from the 75% H_2O-25% EtOH-MC solution and dried at 100C or 80C, can be attributed not only to gel formation that occurred prior to complete dehydration, but also to the disruption of H_2O-MC interactions by EtOH in samples where MC was fully dissolved.

If one compares, at each drying temperature, the influence of the two apparent determinants of crystalline intensity, gelation and EtOH concentration, gelation is found to be the least influential of the two. This is evident when the crystalline intensities of films prepared from a solvent blend of 50% H_2O-50% EtOH are compared with those prepared from water using drying temperatures of either 80C or 100C (Fig. 2). Films prepared from the 50% H_2O-50% EtOH-MC solution did not gel at either of these temperatures, yet they still displayed greater crystallinity than films prepared using water as a solvent. This conclusion is further supported by examining crystalline intensities of all films dried at 50C or at room temperature. Under these conditions, where all film solutions were sols, films prepared from solvents containing EtOH possessed greater crystallinity than films prepared using water as a solvent (same drying temperature; Fig. 2). This was true even though undissolved MC particles were present in films prepared from solutions with high concentrations of EtOH.

In summary, crystalline order in MC films is favored by drying MC

solutions at elevated temperatures, and/or by using a solvent system of water and 25% or less EtOH, solvents that promote gelling prior to complete dehydration.

Oxygen Permeability

The oxygen permeabilities of MC films prepared using water or water-ethanol as solvents and dried at room temperature, are shown in Table 2. The oxygen permeability of water-solvent MC films was 1.82 $g(m \cdot s \cdot Pa)^{-1} \times 10^{-15}$ (25C, 52% RH; Table 2). This value is slightly larger than the value of 1.47 $g(m \cdot s \cdot Pa)^{-1} \times 10^{-15}$ (24C) reported by Grover (1982). However, Grover (1982) did not report the RH at which his film was tested, and a difference in RH could easily cause the discrepancy (Koch *et al.* 1963). Incorporation of a small amount of EtOH (25%) in the film solution caused a significant (α = 0.05) decrease in O_2P as compared to that of films prepared using water as a solvent. However, larger amounts of EtOH caused O_2P to increase to values that did not differ significantly (α = O.05) from that of the water solvent film.

TABLE 2.
OXYGEN AND WATER VAPOR PERMEABILITIES OF METHYLCELLULOSE FILMS[a] MADE FROM SOLUTIONS OF VARIOUS COMPOSITIONS

Solvent Composition (v/v)	Oxygen Permeability[b,c] ($g[m \cdot s \cdot Pa]^{-1} \times 10^{-15}$)	Water Vapor Permeability[c,d] ($g[m \cdot s \cdot Pa]^{-1} \times 10^{-10}$)
100% H_2O	1.82 ± 0.11 AC	0.87 ± 0.08 A
75% H_2O-25% EtOH	1.48 ± 0.11 B	0.77 ± 0.08 A
50% H_2O-50% EtOH	1.66 ± 0.11 A	1.01 ± 0.05 B
25% H_2O-75% EtOH	1.94 ± 0.11 C	1.47 ± 0.08 C

[a] Films were prepared by drying at room temperature (23-25 C) overnight. Resulting films were 0.025 ± 0.005 mm (1.0 ± 0.2 mil) thick.
[b] O_2P tested at 25 C and 52% RH.
[c] Means of four trials ± 95% confidence interval. In a given column, values followed by no common letters differ significantly (α = 0.05).
[d] WVP was tested at 25 C and 52-0% RH.

Since molecular orientation in polymers decreases permeability (Salame and Steingiser 1977), those films with the greatest crystallinity (prepared from solvents containing ethanol and dried at room temperature; Fig. 2) should, and did have, with the exception of the film prepared from the solvent containing 75% EtOH, the smallest permeabilities.

However, the degree of MC crystallinity cannot be responsible for the significant ($\alpha = 0.05$) differences in 0_2P among films containing 25%, 50% and 75% EtOH, because these films possessed equal crystalline intensities (Fig. 2; films dried at room temperature). EtOH is believed to enhance MC-MC hydrogen bonding (Tomioka and Matsumura 1987), and this would tend to strengthen the MC matrix (but not crystallization, which involves hydrophobic associations), encourage molecular association, and lessen O_2P. It is possible that EtOH fulfilled these functions equally well in all EtOH-containing samples, but that the higher levels of EtOH (50%, 75%) resulted in less than full dissolution of MC. If MC solubility decreased with increasing EtOH concentration, as was evident from the appearance of the solutions and films, this would result, with films prepared from solvents containing 50% and 75% EtOH, in poor film integrity and increased O_2P. This would explain the low O_2P of the 25% EtOH film—sufficient EtOH to encourage association of MC but not enough EtOH to lessen the solubility of MC.

Another factor contributing to the large O_2P of the film prepared from a solvent containing 75% EtOH may be an inaccuracy in measuring thickness. This film had an irregular surface which probably resulted in an erroneously large observed value for thickness. If so, the O_2P value, as expressed would be too large.

Water Vapor Permeability

The water vapor permeability of the MC films prepared using water as a solvent was 0.87 $g(m \bullet s \bullet Pa)^{-1} \times 10^{-10}$ (Table 2; 25C, 52–0% RH), and this value is similar to the value of 0.50 $g(m \bullet s \bullet Pa)^{-1} \times 10^{-10}$ (37.8C, approximately 95–0% RH), reported by Grover (1982). Differences between the two values can be attributed mainly to the different test temperatures used (Patel *et al.* 1964). The temperature effect apparently more than compensated for the difference in RH gradient.

The WVPs of films prepared from solvents containing 50% or 75% ethanol were significantly ($\alpha = 0.05$) greater than those of films prepared from solvents consisting of 100% water or those containing 25% ethanol (Table 2). This result is probably due to film discontinuities (undissolved MC) and the erroneous film thickness noted earlier. The WVP of films prepared from solvents of 100% H_20 or 75% H_20-25% EtOH did not differ significantly ($\alpha = 0.05$), even though this was expected because of the structure-altering effects of EtOH as seen in Fig. 2.

Because all films prepared from solutions containing EtOH possessed equal degrees of crystallinity when dried at room temperature (Fig. 2), it is difficult to conclude whether crystallinity has any bearing on the WVPs of these films. Also, when dried at room temperature, the degree of crystallinity in these films was very small and would likely make only a small contribution to the physical properties of the films.

Mechanical Properties

The tensile strength and elongation values of MC films dried at room temperature (23–25C) are shown in Table 3.

Tensile strength of MC films prepared using water as a solvent are consistent with values reported by Greminger and Savage (1959) and Grover (1982), but are slightly greater than those of Ononokpono and Spring (1988). Percent elongation values of the MC films are smaller than those reported by Greminger and Savage (1959), Grover (1982) and Ononokpono and Spring (1988). This discrepancy is likely due to differences in the strain rates used (Aulton 1982).

TABLE 3.
TENSILE STRENGTH AND PERCENT ELONGATION OF METHYLCELLULOSE FILMS CAST FROM WATER/ETHANOL SOLUTIONS[a]

Solvent Composition (v/v)	Tensile Strength[b] (MPa)	Elongation[b] (%)
100% H_2O	71.2 ± 4.7 A	7.3 ± 1.5 A
75% H_2O-25% EtOH	79.5 ± 5.1 B	8.4 ± 1.8 B
50% H_2O-50% EtOH	63.5 ± 5.1 C	5.7 ± 1.6 A
25% H_2O-75% EtOH	36.6 ± 5.6 D	5.8 ± 1.6 A

[a] Films prepared by drying at room temperature (23-25 C) overnight. Resulting films were 0.025 ± 0.005 (1.0 ± 0.2 mil) thick and were conditioned one wk at 52% RH prior to testing.
[b] Means of at least five replicates ± 95% confidence interval. In a given column, values followed by no common letters differ significantly ($\alpha = 0.05$).

Films prepared using a 75% H_2O-25% EtOH solvent displayed the largest tensile strength and greatest percent elongation. This result supports the hypothesis that a relatively low concentration of ethanol in the film solution promotes hydrogen bonding between MC molecules, thus strengthening the matrix and making it more extensible.

The poor tensile strength and elongation of films prepared using 50% H_2O-50% EtOH and 25% H_2O-75% EtOH solvents is probably due to stress fractures caused by MC solids that did not dissolve in these solvents.

The MC films used for this part of the study were all dried at room temperature, so the effect of drying temperature on mechanical properties cannot be assessed. However, Reading and Spring (1984) studied this aspect. They found no significant difference between ultimate tensile strength and percent elongation of MC films prepared from a 100% H_2O solvent and dried at either 20C or 60C. Because MC solutions gel at 60C but not at 20C (close to the 23–25C used here) it would appear that mechanical properties of MC films are not dependent on gel formation (or hydrophobic association leading to crystallization) prior to dehydration.

Contrary to the data of Reading and Spring (1984), increased crystallinity of cellulose fibers generally increases tensile strength and decreases extensibility (Mark 1954). It is difficult to ascertain the effect that crystallinity had on the mechanical properties of the MC films studied here for the reasons mentioned in the section on water vapor permeability.

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THE EFFECTS OF PLASTICIZERS ON CRYSTALLINITY, PERMEABILITY, AND MECHANICAL PROPERTIES OF METHYLCELLULOSE FILMS

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ABSTRACT

The effects of plasticizers (polyethylene glycols [PEG] 400, 1,450, 8,000 and 20,000, glycerol [G] and propylene glycol [PG]), 30% dry basis, on the physical properties of methylcellulose (MC) films were investigated. With the exception of PG, plasticizers with low molecular weights (G and PEG 400) caused the largest increase in the d_{101} spacing of the crystal lattice. All plasticizers significantly ($\alpha = 0.05$) increased oxygen (0_2P) and water vapor permeabilities (WVP) of the films as compared to that of unplasticized MC, with PEG 400 having the greatest effect on 0_2P and G having the greatest effect on WVP. With the exception of PG, all plasticizers decreased the tensile strength of MC films, with PEG 400 causing the largest decrease. With the exception of PG and PEG 400, all plasticizers increased percent elongation values of MC films, with PEG 1,450 having the greatest effect. Glycerol and PEG were the most effective plasticizers for MC. The higher molecular weight plasticizers do provide some plasticizing properties and may be more suitable for applications that require a lower permeability to water vapor than can be achieved with glycerol.

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INTRODUCTION

Methylcellulose (MC) is a cellulose ether formed by alkali treatment of cellulose, followed by reaction with methylchloride (Grover 1982). It exhibits thermal gelation, forms excellent films, and is used in both the pharmaceutical and food industries. The use of MC films in the food industry is, however, not common. Other properties of MC films are discussed in a previous paper (Donhowe and Fennema 1993).

Plasticizers are frequently added to packaging films to enhance pliability. Since plasticizers are believed to decrease polymer interaction and cohesiveness, they would be expected to influence crystallinity and other physical properties of MC films. The effects of plasticizers on the physical properties of films of ethylcellulose (EC) and hydroxypropylmethylcellulose (HPMC) have been studied extensively (Aulton *et al.* 1981; Entwistle and Rowe 1979; Okhamafe and York 1983, 1985; Porter 1980; Sakellariou *et al.* 1986; Tamba Vemba and Roland 1980). However, only a few researchers have investigated the effect of plasticizers on the properties of MC films (Ononokpono and Spring 1987, 1988; Reading and Spring 1984), and reports do not exist on the effects of plasticizers on the crystallinity of MC films or on their permeability to water vapor or oxygen.

Since MC films are of considerable potential importance in foods and the effects of plasticizers on the physical properties of these films are unknown or unpublished, studies in this area are appropriate.

The objectives of this study were to examine the effect of plasticizer type on the crystallinity, oxygen and water vapor permeabilities, tensile strength, and elongation properties of MC films.

MATERIALS AND METHODS

Film solutions were prepared by dispersing the plasticizer in one-half the water (twice distilled, 90C), adding all of the MC (A15 LV; Dow Chemicals, Midland, MI) with continuous agitation, adding the remaining water (10C), and cooling to 10–15C. Film solutions were stored overnight at 5C to help assure complete hydration and to free the solution of visible air bubbles.

Polyethylene glycol (PEG; M_w of 400, 1,450, 8,000, and 20,000 daltons; purity of PEGs not reported by supplier), glycerol (G; ACS reagent grade), or propylene glycol (PG; purity not reported by supplier) were added at a concentration of 30% w/w MC (dry weight). All plasticizers were obtained from Sigma Chemical Co., St. Louis, MO, except for PEG 400, which was purchased from Aldrich Chemical Co., Milwaukee, WI. As indicated in the Code of Federal Regulations (Anon. 1989), G and PG have GRAS status for food use. PEG, with M_w between 200 and 9,500, can be used in all foods, except milk,

as a coating, binder, or plasticizing agent for food tablets (Anon. 1989; CFR 172.820).

Film solutions were cast and dried overnight at room temperature (23–25C) as described by Donhowe and Fennema (1993). Dried films were approximately 0.025 ± 0.005 mm (1.0 ± 0.2 mil) thick. Samples for X-ray diffraction were prepared, stored and examined as previously described (Donhowe and Fennema 1993).

Oxygen permeability (O_2P) and water vapor permeability (WVP) of MC films were determined at 25C using an Ox-tran 100 and a Permatran W1-A instrument, respectively (Modern Controls, Inc., Minneapolis, MN). Details were previously described (Donhowe and Fennema 1993). Unless otherwise noted, at least four replicates of each MC film were tested. A micrometer was used to measure film thickness. Reported thickness values are means of five measurements.

Tensile strength and percent elongation of MC films were determined on at least five film replicates using an Instron Universal Testing Machine, (Model 100, Instron Co., Canton, MA) (Donhowe and Fennema 1993).

Data were initially evaluated using analysis of variance (ANOVA; SAS® Statistical Package; SAS 1985). When the ANOVA test indicated a significant ($\alpha = 0.05$) difference among means, a least significant difference test was used to identify which film means differed significantly.

RESULTS AND DISCUSSION

Qualitative Observations

All plasticizer-MC films, except for those films containing PEG 20,000, were clear after drying. MC films containing PEG 20,000 had a white coating on the surface. A white residue also appeared on the surface of films containing MC-PEG 1,450 and MC-PEG 8,000, but only after these films were stored for more than 6 months.

The appearance of a white residue on the surface of edible films containing plasticizers has been referred to as "blooming" (Aulton *et al.* 1981) or "blushing" (Sakellariou *et al.* 1986). This occurs when the plasticizer concentration exceeds its compatibility limit in the polymer causing phase separation and physical exclusion of the plasticizer (Aulton *et al.* 1981).

MC films containing 30% G, PG, or PEG 400 were clear, indicating that these plasticizers, at the level used, were compatible with MC.

X-Ray Diffraction (XRD) Properties

The XRD patterns of the MC films containing 30% plasticizers were similar to those of comparable films without plasticizers (Donhowe and Fennema

1993), i.e., a sharp peak was evident in the region of 8.25 °2Θ (10.7–11.2 Å, d_{101} spacing), and a broad peak at 20.5–21.5 °2Θ (4.13–4.33 Å, $d_{\bar{1}01}$ spacing). The presence of the sharp d_{101} peak in the diffraction patterns of plasticized MC films confirms the presence of trimethylglucose-type crystalline order in these films (Kato *et al.* 1978; Vasil'ev *et al.* 1973).

The intensity of the d_{101} diffraction peak did not change significantly with the various plasticizers tested. However, the position of this peak was dependent on the molecular weight of the plasticizer added to the film solutions (Fig. 1). Generally, plasticizers with low molecular weights (G and PEG 400) caused the largest increase in the d_{101} spacing. PG was an exception to this pattern. However, even films containing PEG 20,000, in which phase separation occurred, exhibited a significant ($\alpha = 0.05$) increase in crystalline spacing.

Researchers who have examined the interaction between plasticizers and cellulose ethers have concluded that plasticizers interact with amorphous regions of polymers because they decrease the glass transition temperature of the polymer (Entwistle and Rowe 1979; Okhamafe and York 1985; Sakellariou *et al.* 1986). Hydrogen bonding between the plasticizer and MC segments would appear to be most likely in these regions. Plasticizer-induced increases in the mobility of MC segments in amorphous regions probably affect the entire film structure, causing the spacing within crystalline sections to be slightly stretched.

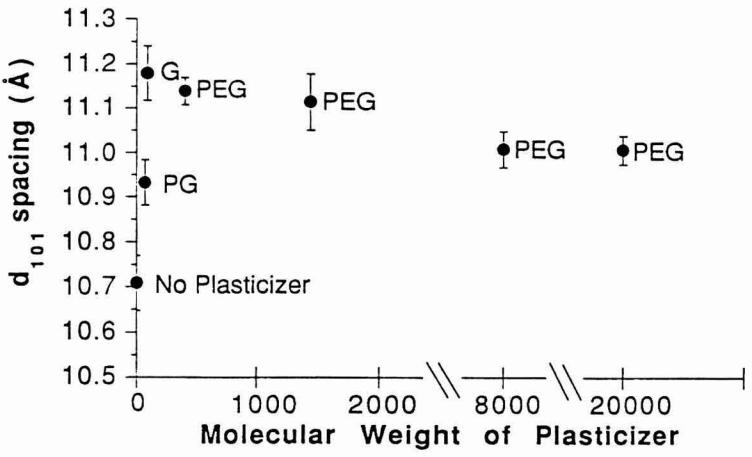

FIG. 1. EFFECT OF GLYCOL DERIVATIVES ON CRYSTALLINITY (d_{101} SPACING) OF METHYLCELLULOSE FILMS

G is glycerol (M_w = 92.1), PG is propylene glycol (M_w = 76.1), PEG is polyethylene glycol (M_w = 400, 450, 8,000, and 20,000). Plasticizer concentration is 30% w/w MC (dry basis). Error bars represent the 95% confidence interval.

The fact that G produced the largest increase in crystalline spacing of the five ethylene glycol derivatives (Fig. 1) is probably attributable to its ability to hydrogen bond with amorphous MC segments. G has a larger number of hydroxyl groups per mole than the high-molecular weight PEGs, and its small size should facilitate penetration of plasticizer into the matrix. PG, another low-molecular weight plasticizer, would be expected to produce an increase in d_{101} spacing comparable to that of glycerol, but it did not (Fig. 1). One reason for the apparent lack of interaction between the PG and MC may be that its polarity (dielectric constant of 32.0 at 20C) is lower than that of G (dielectric constant 42.5 at 25C; Weast 1984). PG may not have the dipole strength to disrupt MC-MC interactions and, therefore, may not be able to position itself effectively between polymer segments.

As the molecular weight of a PEG increases, its polarity decreases (Curme and Johnston 1952), causing an expected decrease in its ability to interact with polymer chains. Nonetheless, sufficient interaction apparently occurred to cause a significant ($\alpha = 0.05$) increase in the d_{101} spacing of MC films.

Oxygen Permeability

The oxygen permeabilities of the plasticized and unplasticized MC films, all dried at room temperature, are shown in Fig. 2. All plasticizers significantly ($\alpha = 0.05$) increased O_2P as compared to that of unplasticized MC. This is not surprising, since plasticization results in increased mobility of polymer chains and, thus, decreased resistance of the film to gas or vapor transmission. Incorporation of PEG 400 in MC films produced the largest increase in O_2P, whereas films containing PG or G had O_2Ps most similar to that of unplasticized MC.

Several factors may be responsible for differences in O_2P of the plasticized MC films. These factors include physical state of the plasticizer, molecular weight of the plasticizer, altered film structure, and chemical interaction between the plasticizer and oxygen.

The high O_2P of the MC-PEG 400 films is likely the result of some of the factors just mentioned. This plasticizer is liquid at room temperature and would likely provide less resistance to diffusion than would the other PEGs, which are solid at room temperature. PEG 400 also created a significant increase in crystalline spacing (as seen in Fig. 1), and this may facilitate diffusion through the expanded film matrix, probably with little compensating ability, because of its moderate compatibility with MC, to fill the expanded matrix in a manner that restricts the flow of O_2.

G, another liquid plasticizer, also would be expected to produce a large increase in O_2P when incorporated in a film. However, as seen in Fig. 2, MC-G films exhibited O_2P only slightly greater than that of unplasticized MC films.

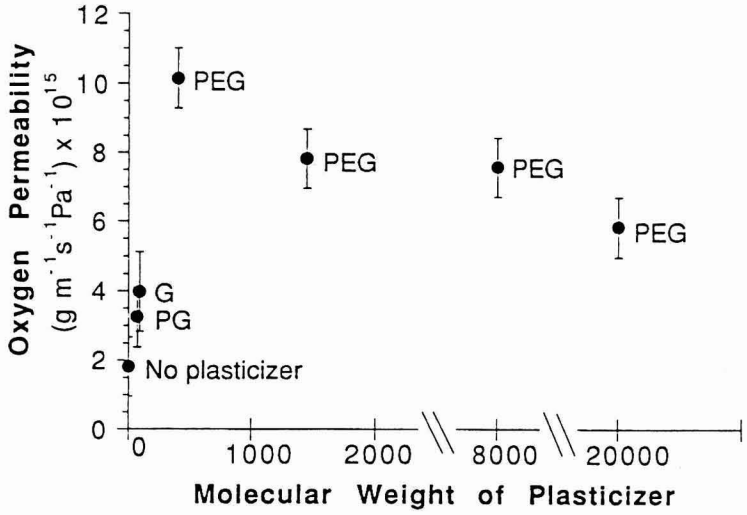

FIG. 2. EFFECT OF GLYCOL DERIVATIVES ON OXYGEN PERMEABILITY OF MC FILMS DRIED AT ROOM TEMPERATURE

G is glycerol (M_w = 92.1), PG is polyethylene glycol (M_w = 76.1), PEG is polyethylene glycol (M_w = 400, 1,450, 8,000, and 20,000). Plasticizer concentration is 30% w/w MC (dry basis). O_2P testing conditions were 25C and 52% RH. Films were 0.025 ± 0.005 mm thick. Error bars represent 95% confidence intervals equalling 0.65 for all films except for G, which equals 0.76. No significant (α = 0.05) difference was found between permeabilities for PG and G, nor PEG 1,450 and PEG 8,000. Permeabilities of all other films were significantly (α = 0.05) different from each other.

Any increase in O_2P that might have been anticipated because of glycerol's fluidity and ability to expand the MC matrix may have been offset by the good oxygen barrier properties of glycerol itself (Koch *et al.* 1963; Müller 1912; Smith 1928), and by the ability of this small molecule to effectively fill small voids in the polymer matrix (Porter 1980).

Although published information was not found on the solubility of oxygen in PG, the reason for the small increase in O_2P of MC-PG films may be similar, at least in one respect, to that for MC-glycerol films, i.e., PG may have effectively filled voids in the polymer matrix.

As the molecular weight of PEG increased, it caused smaller increases in O_2P (Fig. 2), probably due to decreasing interaction between PEG and MC.

Evidence for this possibility comes from the smaller increases in d_{101} spacing caused by PEG as its molecular weight was increased (Fig. 2). Also, the decrease in O_2P with increasing M_w of PEG may be caused by reduced fluidity in the film (PEGs with molecular weights greater than 400 are solid at room temperature). Since no information is available concerning the solubility of oxygen in PEGs, a relationship between molecular weight of PEG and oxygen solubility (which would influence permeability) cannot be established.

Water Vapor Permeability

Similar to the results with O_2P, all plasticizers significantly ($\alpha = 0.05$) increased WVP of MC films (Fig. 3). MC-G films had significantly ($\alpha = 0.05$) larger WVPs than those of the other films. The high WVP of these films can be attributed to the high affinity of G for water, which aids diffusion of water molecules. Porter (1980) found that glycerol (40% w/w) produced the greatest increase in WVP of HPMC films containing many of the same plasticizers used in this study.

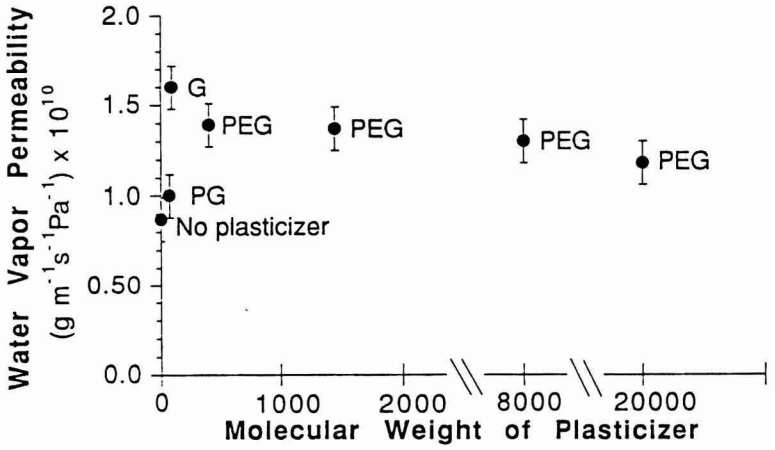

FIG. 3. EFFECT OF GLYCOL DERIVATIVES ON WATER VAPOR PERMEABILITY OF MC FILMS DRIED OVERNIGHT AT ROOM TEMPERATURE

G is glycerol (M_w = 92.1), PG is propylene glycol (M_w = 76.1), PEG is polyethylene glycol (M_w = 400, 1,450, 8,000, and 20,000). Plasticizer concentration is 30% w/w MC (dry basis). Test conditions were 25C, 52-0% RH. Films were 0.025 ± 0.005 mm thick. Error bars represent 95% confidence intervals. Permeabilities of PEG 400, 1,450, and PEG 8,000 films did not differ significantly ($\alpha = 0.05$); however permeabilities of all other films did differ significantly ($\alpha = 0.05$)

Among the PEG-plasticized films, MC with PEG 20,000 possessed the smallest WVP. This likely occurred because the long polyethylene chains of PG 20,000 increased the hydrophobicity of the film, thereby decreasing water solubility. Okhamafe and York (1983) reported that the solubility of water vapor in HPMC-PEG 400 films was greater than its solubility in HPMC-PEG 1,000 films. All of the higher molecular weight plasticizers (PEGs $\geq$ 1,450) are less fluid than the low molecular weight plasticizers, such as G and PEG 400, and this too would likely result in smaller WVP values.

The addition of PG to MC caused a slight but significant (α = O.05) increase in WVP (Fig. 3). The lack of interaction between this plasticizer and MC, discussed in the XRD section, likely caused the increase in permeability to be small. Porter (1980) reported that the addition of PG to HPMC films had practically no effect on WVP.

Data in Fig. 1–3 are consistent with results of Entwistle and Rowe (1979), who reported that interaction between a homologous series of plasticizers and a polymer reaches an optimum at a particular molecular weight and then decreases. This optimum also corresponds to maximum intrinsic viscosity (Entwistle and Rowe 1979). From Fig. 1–3, optimum plasticizer interaction apparently occurs near a molecular weight of 100–400.

Mechanical Properties

The tensile strengths of plasticized and unplasticized MC films are listed in Table 1. The tensile strengths of unplasticized MC and MC-G films in Table 1 are similar to those reported by Greminger and Savage (1959). Except for PG, all plasticizers significantly (α = 0.05) decreased the tensile strength of MC films. Since plasticizers interfere with association of polymer chains, plasticizers would be expected to have this effect.

Greminger and Savage (1959) studied the tensile strengths of MC films containing G, PEG 600, or PG, and found that PG produced the largest decrease in tensile strength, nearly 50%. However, Greminger and Savage (1959) did not provide any information on test conditions.

The largest increases in percent elongation occurred when PEG 400, PEG 1,450, and G were incorporated in MC films (Table 1). There was no significant difference in the percent elongation between MC films containing PG or no plasticizer. Literature values for elongation properties of MC films plasticized with PG vary greatly (Greminger and Savage 1958; Ononokpono and Spring 1987).

PEG 8,000 and 20,000 produced decreases in the tensile strength of MC films that did not differ significantly (α = 0.05) from those produced by other PEGs. However, these compounds were less effective than the other PEGs in increasing percent elongation of the film (Table 1).

TABLE 1.
TENSILE STRENGTH AND PERCENT ELONGATION OF PLASTICIZED FILMS OF METHYLCELLULOSE

Film[a]	Tensile Strength[b] (Mpa)	Elongation[b] (%)
MC	71.2 ± 5.3 A	7.3 ± 3.9 A
MC-Propylene glycol	70.9 ± 5.2 A	11.6 ± 3.9 AB
MC Glycerol	48.6 ± 6.1 BCD	36.7 ± 4.6 C
MC-PEG 400	41.3 ± 5.8 BC	33.0 ± 3.9 C
MC-PEG 1,450	50.3 ± 6.1 BD	41.2 ± 4.6 D
MC-PEG 8,000	43.7 ± 5.2 BCD	17.8 ± 4.2 B
MC-PEG 20,000	45.0 ± 6.1 BCD	13.3 ± 4.6 AB

[a] Films were dried overnight at room temperature and stored 1 wk at 52% RH prior to testing. Films were 0.025 ± 0.005 mm thick. MC is methylcellulose and PEG is polyethylene glycol.
[b] In a given column, values not followed by common letters differ significantly (α= 0.05). Values are means of at least five replicates ± 95% confidence interval.

In selecting a plasticizer for an edible film, the effect on the desired physical properties of the film should be considered. From the physical properties examined in this study, glycerol and PEG 400 appear to be the most effective plasticizers for MC, probably because these plasticizers are compatible, at the 30% level, with MC. In addition, for films exposed only to low RH, glycerol provides beneficial oxygen barrier properties.

MC films containing PEG 1450 have comparable mechanical properties to those containing glycerol or PEG 400, and are less permeable to oxygen. High-molecular weight PEGs provide some desirable plasticizing attributes, such as reduced tensile strengths, which may make them suitable for applications that require better gas or vapor barrier properties that can be achieved from films containing low-molecular weight plasticizers.

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KINETICS AND MECHANISM OF NONENZYMATIC DEAMIDATION OF SOY PROTEIN

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ABSTRACT

Nonenzymatic deamidation of glutamine and asparagine residues of soy protein in aqueous solutions of varying pH (3.0, 5.0, 7.0, 9.0 and 11.0) at different temperatures (100C, 115C and 130C) was monitored over a period of time. The deamidation of soy protein followed first-order kinetics. The activation energies (Ea) of soy protein deamidation at different pHs ranged from 14 to 27 Kcal/mol. The activation energies were highly pH-dependent and decreased very sharply as the pH went up from pH 5 to pH 11.

INTRODUCTION

Recently the effect of chemical and enzymatic deamidation of food proteins on their functional properties has been of great interest to the food industry (Shih 1987, 1990, 1991). Vegetable proteins, such as soy protein and wheat protein, are usually rich in glutamine (Gln) and asparagine (Asn). Whitaker (1977) reported that up to one-third of the total amino acids of cereal proteins is glutamine. Those glutamine and asparagine residues can be either enzymatically or chemically hydrolyzed to glutamic acid and aspartic acid. The deamidated protein has a lower isoelectric point and, therefore, its solubility increases in

Journal of Food Processing and Preservation *17*, 259–268.

many mildly acidic food systems (Finley 1975). It has been reported that levels of deamidation as low as 2–6% could enhance the functional properties of soy and gluten proteins (Matsudomi *et al.* 1982, 1985; Hamada and Marshall 1989).

Nonenzymatic deamidation of proteins has recently been shown to occur during food processing such as twin-screw extrusion of wheat flour (Izzo *et al.* 1993a). They reported that increases in temperature and feed moisture enhanced deamidation and that deamidation was also favored at extremes of pH.

The deamidation reaction during food processing may also have significant effects on the flavor and color development of foods. The ammonia molecule produced from the deamidation reaction can enter into a series of reactions resulting in its combination with various sugar degradation products leading to the formation of amino carbonyls, which produce flavor compounds such as pyrazines (Izzo *et al.* 1993b). These compounds have been widely studied and exhibit very important toasted, baked or nutty notes in foods.

It is, therefore, important to understand the kinetics and mechanism of nonenzymatic deamidation during food processing. The knowledge obtained will hopefully lead to the proper method of controlling the degree of deamidation for producing food products of desired quality.

Several reports discussed the influences of the deamidation process on the functional properties of food protein, such as solubility and emulsifying capacity (Ma *et al.* 1986; Ma and Khanzada 1987; Matsudomi *et al.* 1985). However, no systematic investigation has been done with food proteins to understand what is the dominating mechanism of food protein deamidation under various food processing conditions, and how various factors influence the kinetics and mechanism of this reaction. It was the purpose of this paper to study the effects of pH and temperature on the kinetics of soy protein deamidation in aqueous solutions.

EXPERIMENTAL PROCEDURES

Material

Soy protein isolate (ARDEX DHV) was purchased from Archer Daniels Midland Company (Decatur, IL); the protein content of the isolate was about 91.5% with 0.2% fiber content and 6.0% moisture content according to the manufacturer's analysis. An ammonium chloride standard solution (0.1 M) and an ionic strength adjustment solution for electrode ammonia determination were from Orion Research, Inc. (Boston, MA). Deionized water was used for all reaction mixtures and freshly distilled water was used in all the ammonia determinations.

Ammonia Determination

An electrode method was used for ammonia determination (Shih 1990). A 4 ml aliquot of the deamidation reaction mixture was added to a centrifuge tube that contained an equal volume of 10% trichloroacetic acid (TCA). The mixture was well shaken to make sure all ammonia generated during deamidation dissolved into the TCA solution and all soluble protein precipitated. The mixture was then centrifuged. A 5 ml clear solution from the centrifugation sample was diluted to 100 ml with distilled water before it was analyzed by an ammonia ion-selective electrode (Orion Research, Inc.; Boston, MA). A calibration curve was prepared using standard ammonium chloride solutions (10^{-6} to 10^{-2} M).

Complete Soy Protein Deamidation

In order to determine the total nonpeptide amide content of soy protein isolate, the isolate was subjected to total deamidation according to the method of Shih (1990). Five grams of soy protein were suspended in 100 ml 2 N HCI and the mixture was refluxed for 3 h and then the total ammonia released was measured.

Calculation of Percent Deamidation

$$\% \text{ Deamidation} = (C_{AO}\text{-}C_A/C_{AO}) \times 100\%$$

C_A (m mol/g protein) was the concentration of the remaining amide groups. C_{AO} was the concentration of the initial total amide group at time zero for the deamidation reaction and was determined according to the procedure of Shih (1990) for the complete deamidation reaction. The remaining amide concentration, C_A, after a period time of deamidation was obtained by subtracting the ammonia released from the initial amide concentration, C_{AO}.

Conditions for Kinetics Studies

The following conditions were used for kinetics studies: pH for the reaction mixture: 3.0, 5.0, 7.0, 9.0, 11.0; temperature (C): 100, 115, 130; time for deamidation (h): 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0. One gram of soy protein was suspended in 17 ml distilled water and the pH was adjusted to the required value. The mixture was then transferred into an 18 ml Kimax Brand glass tube with a black phenolic screw cap in which there is a PTFE-coated temperature and pressure resistant rubber liner (Fisher Scientific, Piscataway, NJ). In order to further prevent ammonia vapor loss, an additional 1 mm thick and temperature resistant PTFE-coated silicone rubber flat disc septa (Fisher Scientific, Piscataway, NJ) was placed into the cap to secure the air tightness of the reaction tube. The reaction tubes were then placed in an oil bath at the required

constant temperature. The tubes were regularly shaken to ensure uniform reaction of the heterogeneous protein suspension. Samples were withdrawn at required time intervals and the reaction ceased when samples were immersed in ice water. All kinetic studies were carried out in duplicate and all samples were tightly caped and stored in a freezer before ammonia analysis was conducted. The data were analyzed using a linear regression.

RESULTS AND DISCUSSION

Experimental data on the overall deamidation reaction of soy protein solution were fitted with zero-order, first-order and second-order methods. As shown in Fig. 1–3, the reaction followed an apparent first-order reaction. The R^2s of first-order regression were in the range of 0.968 to 0.999. Although the kinetics of protein deamidation have not been studied previously, Patel and Borchardt (1990a) have reported a pseudo-first-order deamidation reaction of an Asn-hexapeptide in an aqueous solution at 37C.

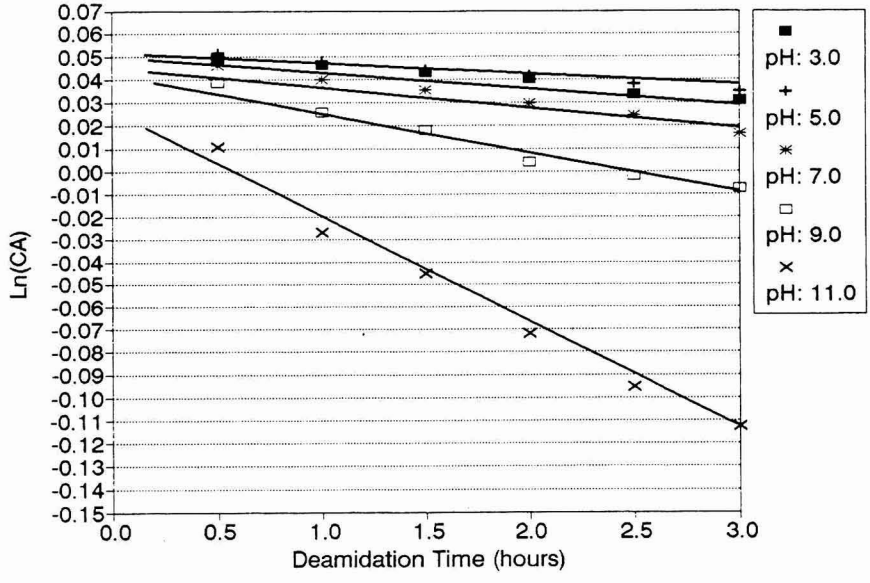

FIG. 1. APPARENT FIRST-ORDER DEAMIDATION OF SOY PROTEIN AT VARYING PH AND 100C

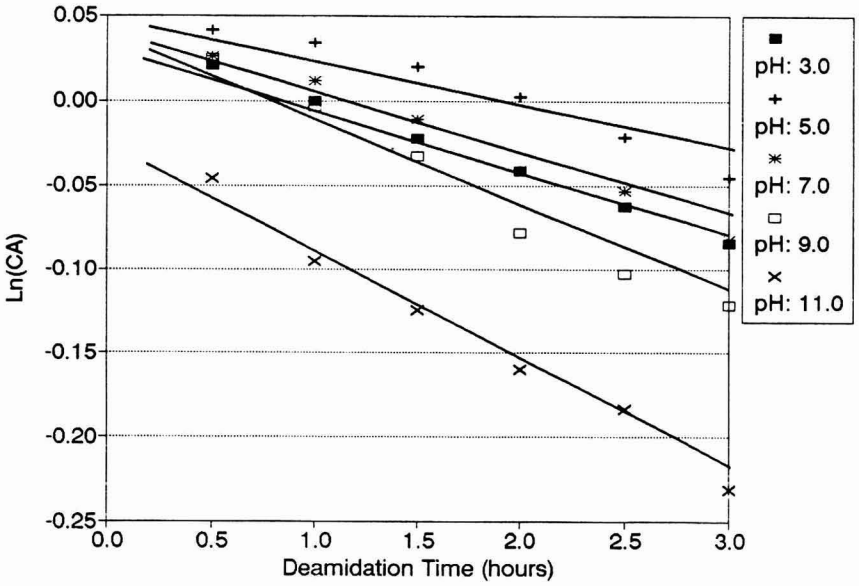

FIG. 2. APPARENT FIRST-ORDER DEAMIDATION OF SOY PROTEIN AT VARYING PH AND 115C

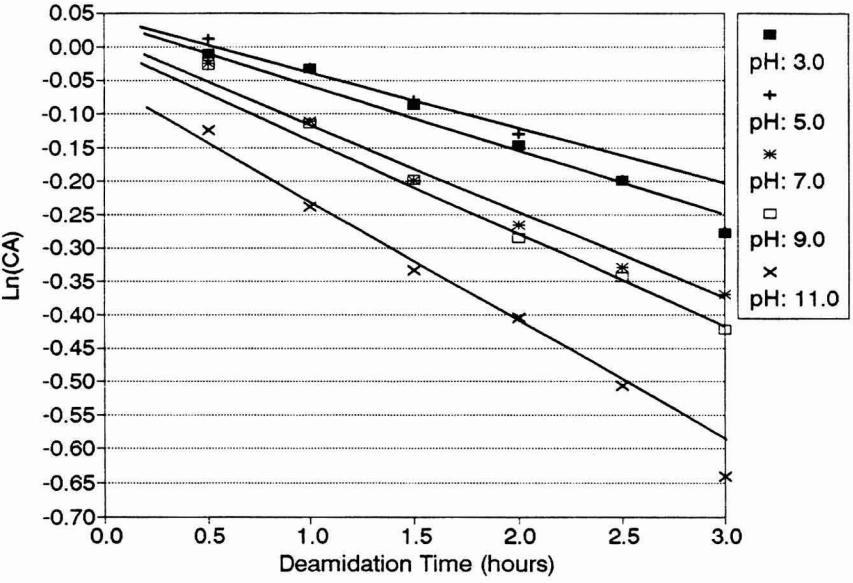

Fig. 3. APPARENT FIRST-ORDER DEAMIDATION OF SOY PROTEIN AT VARYING PH AND 130C

The rate constants of soy protein deamidation at different pHs and temperatures were calculated by linear regression using first-order kinetics (Fig. 4). The results show that the rates of soy protein deamidation were influenced by the pH of the solution. Under all three experimental temperatures, the deamidation reaction exhibited a minimum reaction rate at about pH 5.0. Using model peptides, other researchers also observed the pH minimum phenomena at slightly acidic conditions. Scotchler and Robinson (1974) reported a pH minimum for deamidation of Gly-Leu-Gln-Ala-Gly and Gly-Arg-Gln-Ala-Gly at about 6.0 for both peptides. When studying deamidation of a Asn-hexapeptide (L-Val-Tyr-L-Pro-L-Asn-Gly-L-Ala), Patel and Borchardt (1990a,b) also observed a pH minimum for the reaction rate at about 3.5 and proposed that the ionizable side chains had different ionization states, which might have different catalytic effects, depending on the pKa of individual ion species. The overall reaction rates of peptide deamidation were, therefore, controlled by the total hydronium ion concentration over the experimental pH ranges.

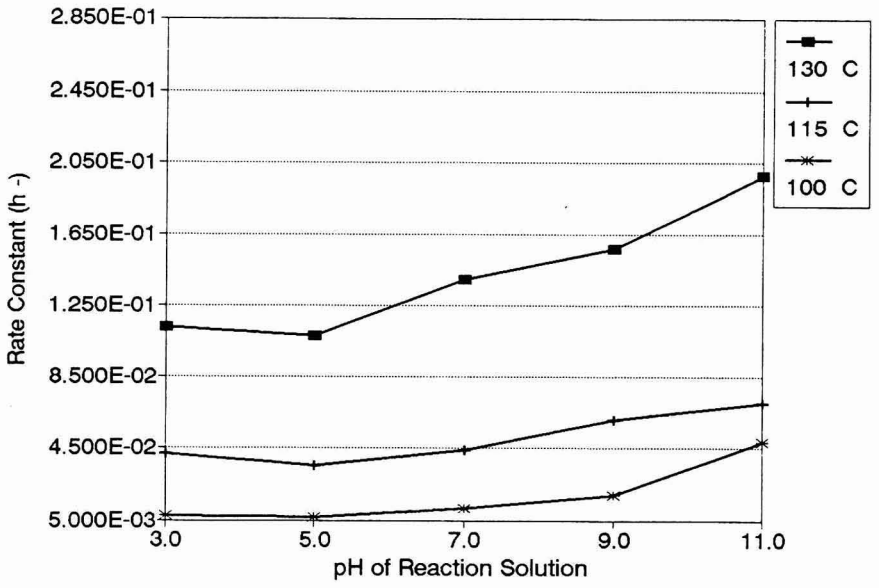

FIG. 4. EFFECTS OF PH ON RATE CONSTANTS OF DEAMIDATION AT THREE DIFFERENT TEMPERATURES

The soy protein had the lowest solubility around pH 4–5 (Snyder and Kwon, 1987), and the solubility increased dramatically either above pH 5 or below pH 4. It is possible that the solubility of soy protein, which is controlled by the charges and three dimensional structure of protein, could directly influence the rates of soy protein deamidation.

The Arrhenius plot is shown in Fig. 5 and the activation energies (Ea) of soy protein deamidation at different pHs are shown in Table 1. They ranged from 14 to 27 Kcal/mol. It is worth noting that the difference in activation energies of soy protein deamidation between pH 3 and pH 5 was very small. However, the activation energies of deamidation reaction decreased very sharply as the pH went up from pH 5 to pH 11. This indicated that the mechanism for the deamidation of soy protein in acidic conditions was different from that under the basic conditions.

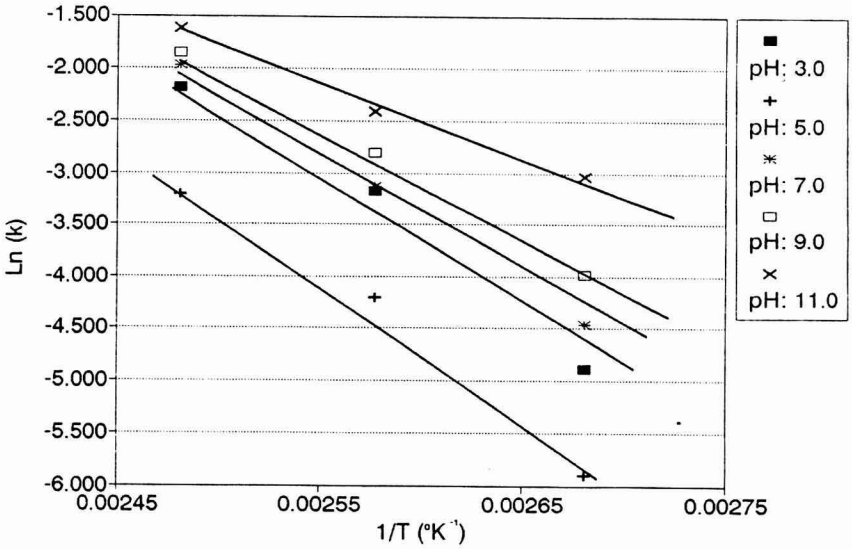

FIG. 5. ARRHENIUS PLOT AT VARYING PH ON SOY PROTEIN DEAMIDATION

TABLE 1.
EFFECT OF pH ON ARRHENIUS ACTIVATION ENERGIES OF SOY PROTEIN DEAMIDATION

pH	3.0	5.0	7.0	9.0	11.0
E_a (Kcal/mol)	26.8	27.0	24.9	21.1	13.9

Differences in rates and mechanism of deamidation of model peptides under acidic and basic conditions have been studied by several researchers (Stephenson and Clarke 1988; Lowenson and Clarke 1988; McFadden and Clarke 1987; Clarke 1987). Geiger and Clarke (1987) reported that the deamidation of asparaginyl residues in protein under physiological conditions was initiated by the formation of a five-membered cyclic succinimide intermediate. Lura and Schirch (1988) later confirmed the five-membered ring mechanism by using ^{13}C and ^{1}H NMR spectroscopy and reported that local protein backbone conformation had a critical influence on the deamidation rate of the individual asparaginyl group. It was postulated (Wright 1991) that Gln residues may also undergo deamidation reactions via the formation of a six-membered cyclic imide at a slower rate than that of Asn residues. This is expected because there is a greater distance from the adjacent peptide amide -NH- groups to the Gln side-chain amide group than that of the Asn side-chain amide group. Patel and Borchadt (1990a,b), using a hexapeptide model system, confirmed that deamidation of the -Asn-Gly- sequence in the peptide at neutral to alkaline pH's involved a cyclic imide intermediate which hydrolyzed to yield the isoAsp and Asp peptides. On the other hand, they observed that the deamidation of the Asn-containing peptide at acidic conditions involved mainly the direct hydrolysis of the side-chain amides rather than via the formation of the cyclic imide intermediate. The rapid deamidation rates for soy protein in basic solution were consistent with the report of Capasso *et al.* (1989) who studied the deamidation of Boc-Asn-Gly-Gly-NH_2. They reported that the formation of the cyclic intermediate was the rate-limiting step of the overall deamidation. The hydrolysis of cyclic imide intermediate was fast reaction. They also found that the rates of both cyclization and hydrolysis reactions increased significantly when the pH of the solution increased.

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HEAT TRANSFER RATES IN A CANNED FOOD MODEL AS INFLUENCED BY PROCESSING IN AN END-OVER-END ROTARY STEAM/AIR RETORT

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ABSTRACT

The effect of various factors on the heat transfer rates of a canned food model (gelatinized starch) were evaluated during thermal processing in an agitating retort. Process variables were retort temperature (110–130C), rotation speed (10–20 rpm), can headspace (6.4–12.8 mm) and starch concentration (3–4%). Thermal process parameters (heating rate index, f_h, and heating lag factor, j_{ch}) and the resulting process lethality, F_o and cook value, C_o were obtained from time-temperature data obtained during processing for three consecutive runs. Rheological properties and color were experimentally evaluated both before and after each run. Overall heat transfer coefficient (U_o) was calculated from the heating rate index using a lumped capacity approach. The study indicated that f_h, j_{ch}, F_o and C_o were influenced ($p < 0.05$) by all process variables except can headspace. U_o was related to retort temperature, rotation speed and initial apparent viscosity. The best dimensionless correlation for U_o was Nusselt number (logarithmic scale) versus specific apparent viscosity and Froude number ($R^2 = 0.65$).

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INTRODUCTION

Container agitation in rotary retorts during processing offers several advantages over still-retort processing, especially improved quality and reduced process times as a result of increased rates of heat penetration. Rotational retorts have been commercially employed in the production of high quality peas, corn, asparagus, mushroom and a variety of semi-solid or viscous foods such as sauces and soups containing meat chunks or vegetables (Eisner 1988). Due to shorter process times required, rotary sterilization utilizes less energy and allows for more productivity as well as improved quality of viscous foods, especially in large containers. Prediction of temperature history of food particles during an agitated cook is a complex phenomena. New products, packages and processes demand thorough testing and predictable performance during processing to benefit from the faster process while assuring the required minimum lethality.

The early research (Ball 1938; Clifcorn *et al.* 1950; Nicholas *et al.* 1960) have demonstrated can agitation during heating to be effective as a means of increasing the rate of heat penetration in cans. The above studies, in general, suggest that the can headspace, solid-liquid ratio, consistency of the product, and the speed and orientation of agitation are important factors to be standardized in agitated processing. Nicholas *et al.* (1960) reported that agitation also prevents separation of different ingredients in the product during thermal processing as in the case of water and syrup systems, which tend to stratify.

The majority of studies on rotational processing (Clifcorn *et al.* 1950; Conley *et al.* 1951; Parchomchuk 1977; Houtzer and Hill 1977; Berry *et al.* 1979; Berry and Bradshaw 1980, 1982; Berry and Dickerson 1980; Berry and Kohnhorst 1985) deal with the effect of agitation on product specific heat penetration parameters. The more recent studies have focused on the fluid to particle heat transfer coefficient, which is an important parameter influencing the heating rate of food particles (Lenz and Lund 1978; Naveh and Kopelman 1980; Soule and Merson 1985; Anantheswaran and Rao 1985a, b; Rao *et al.* 1985; Deniston *et al.* 1987).

The objectives of this study were to evaluate the effects of retort temperature, rotation speed, can headspace and starch concentration on product heat transfer rates, lethality, cook value and physico-chemical properties (viscosity and color) of a canned food model (gelatinized starch). A secondary objective was to develop predictive correlations for heat transfer and quality parameters.

MATERIALS AND METHODS

Food Model

Therm-Flo Starch (Lot #EA 4767, National Starch and Chemical

Corporation, Bridgewater, NJ), a cross-linked waxy maize starch with commercial applications in several food preparations (sauces, gravies, puddings), was used to prepare samples of gelatinized 3% and 4% starch solutions. The two starch concentrations were selected to approximately simulate the product consistency of commercial soups and sauces. The starch suspension was boiled in a steam jacketed kettle for 45 min followed by slow simmering for 2 h to permit starch gelatinization. Water was added to compensate for evaporative losses and to ensure uniform starch concentrations both before and after each experimental run.

Thermal Processing

The gelatinized starch solutions were then hot filled (80C) into 307 × 409 cans allowing for two levels of headspace: 6.4 mm (8/32 in.) and 12.8 mm (16/32 in.). The cans were then placed in a steamer to ensure adequate formation of a vacuum before being sealed in a seaming machine (Double Seamer, Type W-200, Continental Can Co., New York). The cans were stored at 4C overnight before retorting.

Heat penetration tests were conducted in a Lagarde Rotary Simulator (Autoclaves Lagarde, Montelimar, France) retort with a heating medium that consisted of a 75% steam / 25% air mixture. The simulator consisted of a horizontal shell (diameter, 52.1 cm) and a rotary cage (27.5 cm height × 29.5 cm width × 57 cm length) in which the space along the central axis was reserved for positioning open cans with installed thermocouples for measuring the retort temperature. Test cans were located along the periphery nearly equidistant from the central axis (four cans in given vertical cross section). Due to the symmetrical nature of the cage, all test cans were assumed to receive a somewhat similar rotary agitation. Dummy cans were used as required to fill the retort. Temperature readings were computer recorded at 15 s intervals via a data logger (Dash-8, Metra-Byte Corp., Tauton, MA) as cans were subjected to end-over-end rotation. A 3 × 3 factorial experimental design was employed with three retort temperatures (110, 120 and 130C) and three rotation speeds (10, 15 and 20 rpm). Each run consisted of 10 min come-up, 30 min process and 25 min cooling time.

For each test, several cans containing CNS copper-constantan needle type thermocouples (Ecklund-Harrison Technologies, Cape Coral, FL) with tips located at the geometric center were used to gather heat penetration data. Thermocouple accuracies were better than ± 0.5C. Each test run was repeated twice after removing sample cans for property evaluations (viscosity and color), while the thermocouple-equipped cans were left in the retort to gather time-temperature data from the repeated processing. Repeated processing was originally carried out to get additional data under similar processing conditions.

However, it was realized that this procedure would not constitute triplication of the process, since the associated viscosities of the product increased to some extent following each test run. The results, therefore, only served to provide additional data at different apparent viscosities under each of the nine processing conditions employed in this study.

Rheological Properties

A Haake rotational viscometer Model RV20 (Haake Hess-Technik GmbH u. Co., Karlsruhe, Germany) was used to evaluate the rheological properties of the starch solutions both before and after each test run. Test samples equilibrated to 25C were sheared at a programmed rate linearly increasing from 0 to 200 s^{-1} in the first 4 min and then decreasing from 200 to 0 s^{-1} in the next 4 min. The resulting flow curves were fitted by the power law model as shown below:

$$\sigma = m\dot{\gamma}^n \tag{1}$$

Although rheological behavior was characterized by the m and n values, an apparent viscosity value, η, was also evaluated as a ratio of $\sigma/\dot{\gamma}$ at the mid-shear rate of 100 s^{-1} and used as a parameter for the predictive modeling of heating rates. In order to use in dimensionless correlations, a specific apparent viscosity was defined, which is apparent viscosity of product divided by the viscosity of water. The product apparent viscosity was based on m and n values and speed of rotation as suggested by Rao and Anantheswaran (1988):

$$\eta_{sp} = [m\ 8^{n-1} / (N/60)^{1-n}]\ [(3n+1)/(4n)]^n / \eta_{water} \tag{2}$$

Color Measurement

Tristimulus color values (Hunterlab L, a and b values) of test samples were measured both before and after processing using a Minolta Chroma Meter (Minolta Corp., Ramsey, NJ). The total color difference (Delta E, ΔE) was obtained using Eq. (3) (Francis and Clydesdale 1975):

$$\Delta E^2 = [\Delta L^2 + \Delta a^2 + \Delta b^2] \tag{3}$$

Data Processing

Thermal Process Parameters. Since small differences in initial and retort temperatures were unavoidable, data were normalized to an initial temperature of 20C and respective set point retort temperatures (110, 120 and 130C) according to procedures detailed in Stumbo (1973). Heating rate index, f_h and lag factor, j_{ch} using a 42% effectiveness for the 10 min come-up period were

evaluated using established procedures (Stumbo 1973).

Process Lethality. Process lethality (F_o value during the entire process) was calculated for each processing condition by numerical integration of time-temperature data (z = 10 C°):

$$F_o = \int 10^{(T-121.1)/z} dt \quad (4)$$

Cook Value. A "cook value" has been generally defined as equivalent minutes of heating at 100C (Eisner 1988). In order to compute this, again the numerical integration approach was used with a z value of 33 C°, which represents an average z for typical food quality factors (color, texture, nutrients):

$$C_o = \int 10^{(T-100)/33} dt \quad (5)$$

The cook value represents "cooked quality" of the processed product and hence, in order to optimize product quality, it is desired to keep C_o as low as possible while assuring the minimum F_o. For equivalent lethality processes (all having same resulting F_o), the cook value offers a convenient means of comparing the degree of cooking. When the process lethalities of test runs are not constant, as in the present study with a fixed 30 min process, the ratio C_o/F_o would give a relative parameter for comparing the degree of cooking at various retort temperatures with higher ratios indicating more severe cooking conditions (Mohr and Kirchstein 1988; Mohr and Skrok 1990). These ratios yield C_o values when multiplied with the desired F_o.

Overall Heat Transfer Coefficient. The overall heat transfer coefficient was obtained using the perfect mixing convection heat transfer model as reported elsewhere for similar applications (Deniston *et al.* 1992). Heat transferred across the can wall at any given time will thus be equal to the heat absorbed by the product resulting in an increase in its bulk temperature. The overall heat transfer coefficient (U_o) can then be obtained using Eq. (6) (Deniston *et al.* 1992):

$$U_o = 2.303\ m_p\ C_p/(f_h A) \quad (6)$$

The above equation has also been used to evaluate the prevailing surface heat transfer coefficient (h) under the slower conduction type heat transfer using the lumped capacity approach (Kreith 1965) when the associated Biot number is low (<0.1). As shown elsewhere (Ramaswamy *et al.* 1983), under conditions where the Biot number exceeds 0.1, the associated h values will be generally larger with those calculated using Eq. (6) representing a conservative value.

Dimensionless Correlations. An attempt was made to develop the correlation between overall heat transfer coefficient and factors influencing it in terms of dimensionless numbers. The first attempt was to try the conventional correlation: logarithm of Nusselt number (Nu) with logarithm of Generalized Reynolds number (GRe) and Generalized Prandtl number (GPr). The apparent viscosity for use in these correlations was calculated at a shear rate characteristic to the rotation speed employed (Rao and Anantheswaran 1988). The characteristics dimension used was the diameter of rotation. Since the speed of rotation was an important parameter in rotational processing, Froude number (Fr), which is the ratio of inertial forces to gravitational forces (Loncin and Merson 1979), was also employed as the dimensionless speed of rotation. Other properties required for computing the dimensionless numbers are detailed in Awuah *et al.* (1993).

RESULTS AND DISCUSSION

Heat Penetration Parameters

Typical semi-logarithmic heat penetration curves for canned 3% and 4% starch during the first (Run 1) and third (Run 3) time processing at 120C and 15 rpm rotation speed are shown in Fig. 1. As indicated, these curves demonstrate a strong characteristic lag period, not typical of usual convection heating in which no significant lag period exists. The induction of forced convection and mixing of the contents are apparent only after the lag period. This initial viscous resistance of starch samples is perhaps overcome by the continued shearing and heating of the product.

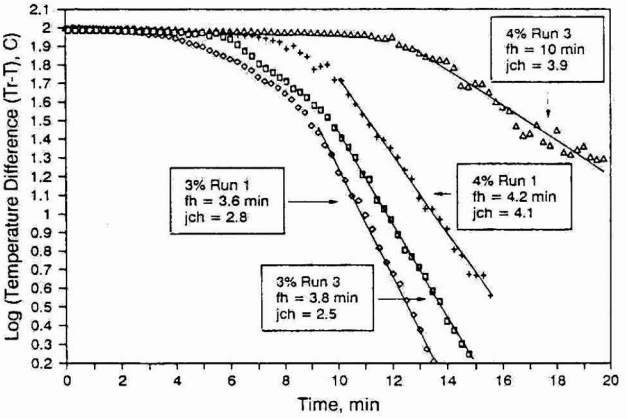

FIG. 1. SEMI-LOGARITHMIC HEAT PENETRATION CURVES FOR 3% AND 4% STARCH IN CANS PROCESSED AT 120C IN A ROTARY AUTOCLAVE AT 15 RPM DURING FIRST AND THIRD RUNS

Analysis of variance of data on f_h and j_{ch} during the ~35 min process (including the effective portion of come-up period) and the accumulated overall F_o and C_o (Table 1) revealed that the headspace influence between 6.4 mm (8/32") and 12.8 mm (16/32") on all these parameters was generally small ($p > 0.05$) and inconclusive. All other factors (starch concentration, temperature and rotation speed) significantly ($p < 0.05$) influenced the parameters.

Heating Rate Index. Concentration of starch had the most significant effect on the heating rate index, accounting for approximately 60% of the total variation (Table 1). Rotation speed was next (15%) followed by temperature (9%). The distribution of f_h with data pooled up with respect to headspace is shown in Fig. 2 for all the three runs. As expected, in general, f_h was found to increase with starch concentration (being higher for 4% starch gel as compared to 3%) and decrease with increasing rotation speed. The effect of starch concentration on f_h could be explained based on the apparent viscosity, which was higher with 4% starch as compared with 3%. The apparent viscosity also varied from sample to sample causing some scattering of f_h values. The decreasing effect on f_h of rotation speed obviously results from the forced mixing of the contents. Figures 1 and 2 also show the effect of repeated processing: a general increase in f_h as the process is repeated (especially during the third). The temperature effect was somewhat mixed with the heating rate index at 120C (mid temperature) being generally lower than at the other two temperatures. Since no specific explanation was possible, it was initially concluded that this observation may have been an artifact.

TABLE 1.
ANALYSIS OF VARIANCE FOR FACTORS INFLUENCING HEATING RATE INDEX(f_h), HEATING LAG FACTOR (j_{ch}), PROCESS LETHALITY (F_o) AND COOK VALUE (C_o)

	Influencing Factors			
Main Effects	f_h	j_{ch}	F_o	C_o
	%SS	%SS	%SS	%SS
Starch Conc.	58.64^{**}	53.78^{**}	2.98^{**}	7.69^{**}
Headspace	$1.08^{n.s}$	$0.30^{n.s}$	$0.02^{n.s}$	$0.07^{n.s}$
Temperature	8.96^{*}	9.10^{*}	87.6^{**}	83.6^{**}
Rotation	14.84^{**}	9.19^{*}	1.3^{*}	2.0^{*}

** $p \leq 0.01$
* $p \leq 0.05$
n.s $p > 0.05$
%SS Percentage sum of squares

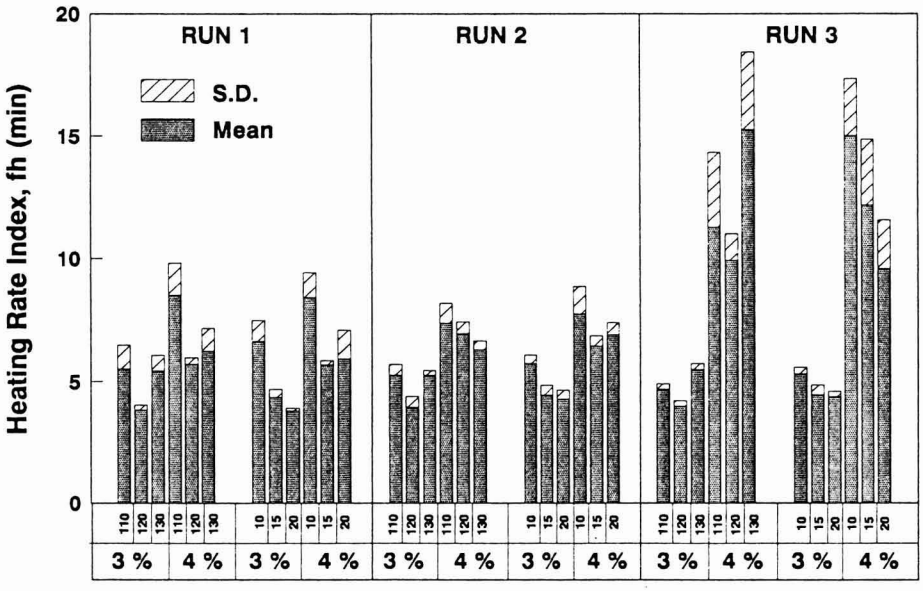

FIG. 2. HEATING RATE INDEX AS INFLUENCED BY TEMPERATURE (110, 120 AND 130C) ROTATION SPEED (10, 15 AND 20 RPM) AND STARCH CONCENTRATION (3% AND 4%) FOR ALL THREE RUNS

Lag Factor. Starch concentration was also the major factor contributing to 54% of total variability (Table 1) with respect to the heating lag factor (j_{ch}). As with heating rate index, temperature and rotation speed came next, each accounting for approximately 9% of total variability. As expected, the lag factor increased with starch concentration (Fig. 3). The temperature effect was mixed as observed with the heating rate index. However, contrary to expectation, the lag factor also increased with an increase in rotation speed. This most likely occurred as a result of resistance to mixing of the starch solutions at the beginning (presence of characteristic lag period) of the heating process. This behavior was also responsible for the sometimes unusually high j_{ch} values greater than the theoretical maximum (2.06) even for conduction heating finite cylinders with a zero come-up period. With induced forced convection, the j_{ch} values should be expected to be lower than for conduction heating (for perfect mixing j_{ch}). The higher j_{ch} values also suggest that the conventional come-up period correction factor of 42% (on which j_{ch} is dependent) is perhaps too conservative for use in rotational processing, especially when involving viscous products.

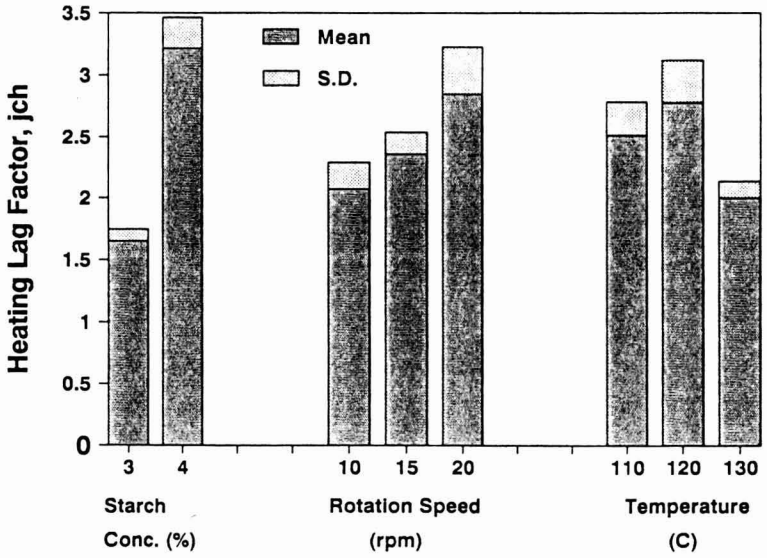

FIG. 3. HEATING LAG FACTOR AS INFLUENCED BY STARCH CONCENTRATION (3% AND 4%), ROTATION SPEED (10, 15 AND 20 RPM) AND TEMPERATURE (110, 120 AND 130C)

Process Lethality and Cook Value

As previously mentioned, accumulated process lethality (F_o) and cook values (C_O) were also influenced ($p<0.05$) by all factors except can headspace (Table 1). With respect to these two factors, temperature was the most dominant factor, accounting for more than 80% of total variability. On an average, F_o was ~2 min at 110C, ~17 min at 120C and ~180 min at 130C (Fig. 4). The cook values similarly varied from 54 min at 110C to 104 min at 120C and 217 min at 130C.

The F_o value of a process indicates its severity with respect to microbial destruction and is the key in process time establishments. The C_o or cook value, on the other hand, indicates the cumulative equivalent of cooking minutes at 100C. The factor C_o/F_o could thus be a suitable multiplier, which when used with the process lethality gives a relative measure of the degree of cooking. Figure 5 shows a response surface plot of C_o/F_o as a function of temperature and rotation speed for 4% starch solution. The ratio dramatically decreases from

about 35 to 2 as the temperature is increased from 110 to 130C. The influence of rotation speed was small as compared with the temperature effect, but the trend was clear. Higher rotation speeds resulted in a decrease in the C_o/F_o. A similar plot of C_o values for time-adjusted processing (to get F_o of 10 min) is given elsewhere (Abbatemarco and Ramaswamy 1992), indicating almost identical trends. It is evident that the cook quality of the processed product can be considerably improved by high temperature short time processing.

Rheological Changes

While heat penetration parameters of the canned starch model during rotational processing were dependent on its initial apparent viscosity, the heating process also had an effect on the product viscosity. The apparent viscosity values were found to increase following each run. The resulting viscosity was generally dependent on the severity of the heat process, high temperatures and high rotation speeds contributing to larger viscosity build-up (process time being the same). The resulting viscosity showed an excellent correlation ($R^2 = 0.94$) with the initial viscosity and cook value of the process.

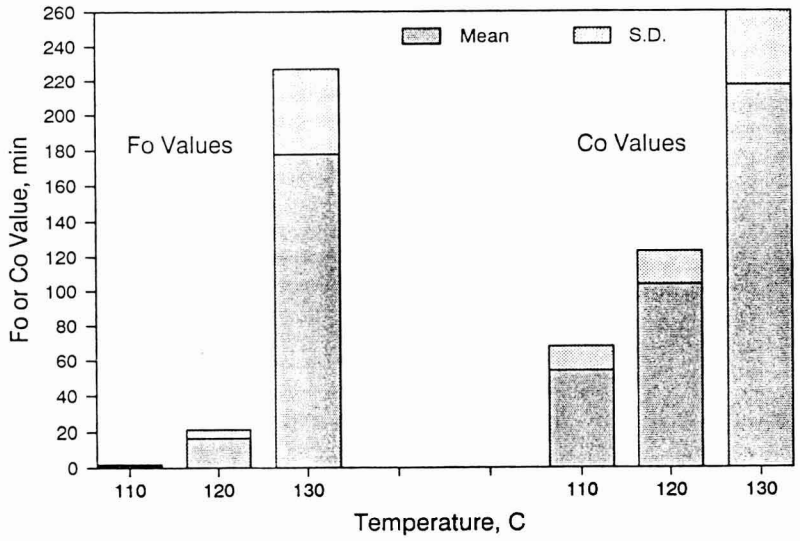

FIG. 4. PROCESS LETHALITY AND COOK VALUE AS INFLUENCED BY TEMPERATURE (110, 120 AND 130C)

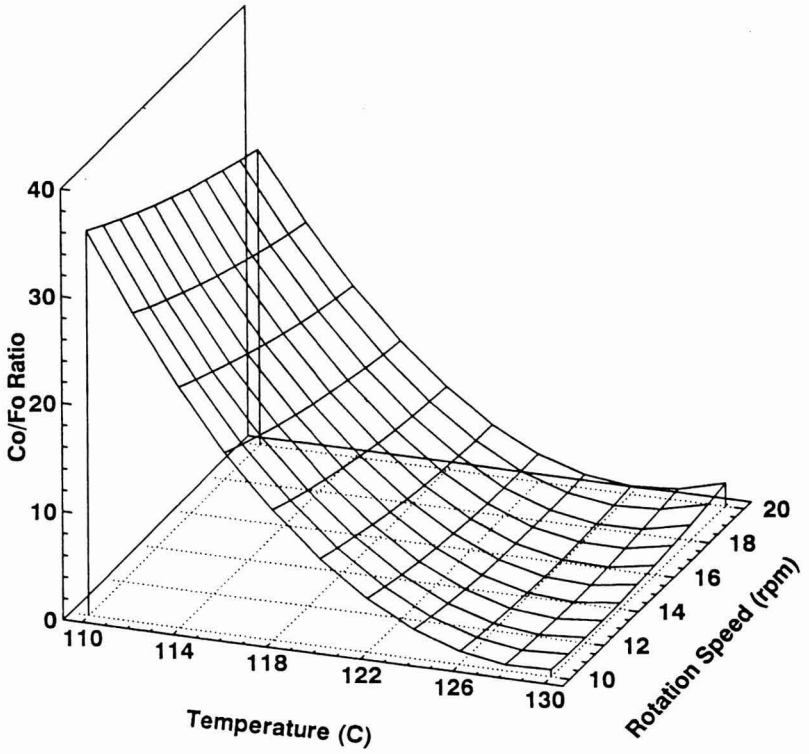

FIG. 5. RESPONSE SURFACE PLOT FOR C_0/F_0 RATIO (4% STARCH) AS INFLUENCED BY TEMPERATURE AND ROTATION SPEED DURING THE FIRST RUN

Color Changes

Hunterlab L, a and b values were influenced by rotation speed and temperature. Since the changes in the individual values were small, the total color change represented by ΔE was used as a combined parameter. ΔE values were mostly influenced ($p < 0.01$) by retort temperature and rotation speed (Fig. 6) somewhat in consistence with the severity of the heat process increasing with temperature and rotation speed. In fact, when all three runs are considered, the greatest total color change was observed at the highest temperature and rotation speed employed in this study, which is the most severe condition. Visual color changes could thus provide objective measures of the degree of cooking.

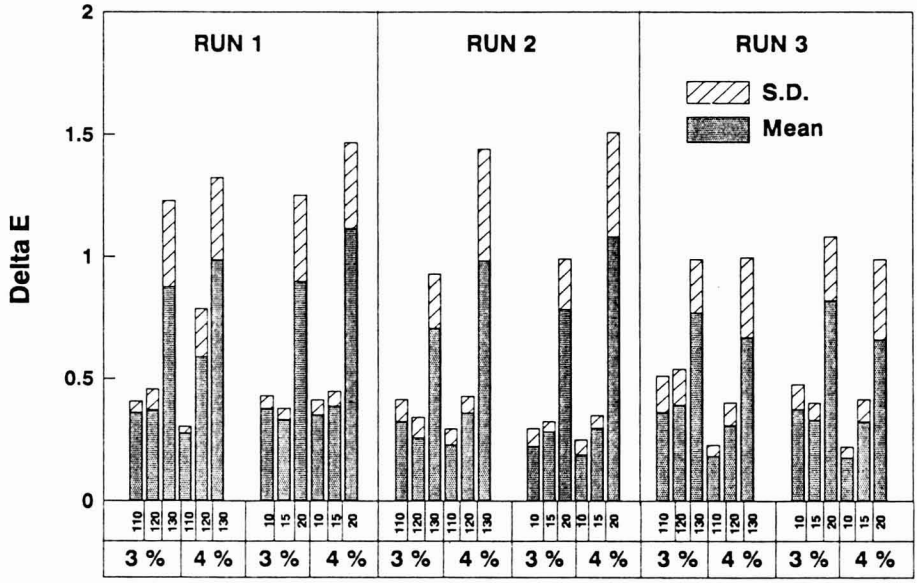

FIG. 6. TOTAL COLOR DIFFERENCE ΔE, DELTA E) AS INFLUENCED BY TEMPERATURE (110, 120 AND 130C), ROTATION SPEED (10, 15 AND 20 RPM) AND STARCH CONCENTRATION (3% AND 4%) FOR ALL TTHREE RUNS

Overall Heat Transfer Coefficient

Under perfect mixing situations caused by container agitation, as in the case of low viscosity fluids, temperature gradients within the can become negligible and the overall heat transfer coefficient U_o calculated using Eq. (6) can be expected to match the surface heat transfer coefficient at the container-product interface. In the present study, test data from first runs (with multiple thermocouples attached to each can) generally revealed internal temperature gradients of less than 1C at any given time. However, as the viscosity of the sample increased, as in repeated second and third runs, temperature gradients within the can became more dominant during the lag period, tending to converge toward the end of the process. Under these situations, Eq. (6) was assumed to yield an estimate of the minimum surface heat transfer coefficient, with the product providing a significant barrier. A similar approach was used by Deniston *et al.* (1992) to determine the overall heat transfer coefficients associated with heating of canned tomato concentrates in a Steritort (with f_h values in similar containers ranging from 5–15 min).

The effect of starch concentration, rotation speed and temperature on the overall heat transfer coefficient is shown in Fig. 7. An increase in rotation speed generally resulted in an increase in U_o for all three runs and for both 3% and 4% starch solutions. The temperature effect again showed higher U_o values at the intermediate temperature.

Since the temperature effect on every parameter tested showed this type of change at the intermediate temperature of 120C, it was presumed that this must be due to some property associated with the waxy maize starch used in the study. Some earlier results on waxy maize starch (Swinkels 1985) revealed that this specialty starch, recommended by the manufacturers for high temperature applications, will not fully solubilize unless heated to temperatures exceeding ~125C, which might provide some explanation to the observed behavior. While the conventional behavior of viscosity thinning can account for increased U_o values between 110 and 120C, the increased viscosity due to continued gelatinization can explain the lowering of U_o at 130C. It should be recognized that a certain procedure was followed to pregelatinize the starch prior to filling in cans. However, as evidenced by increased viscosity following each run, the gelatinization process was obviously incomplete.

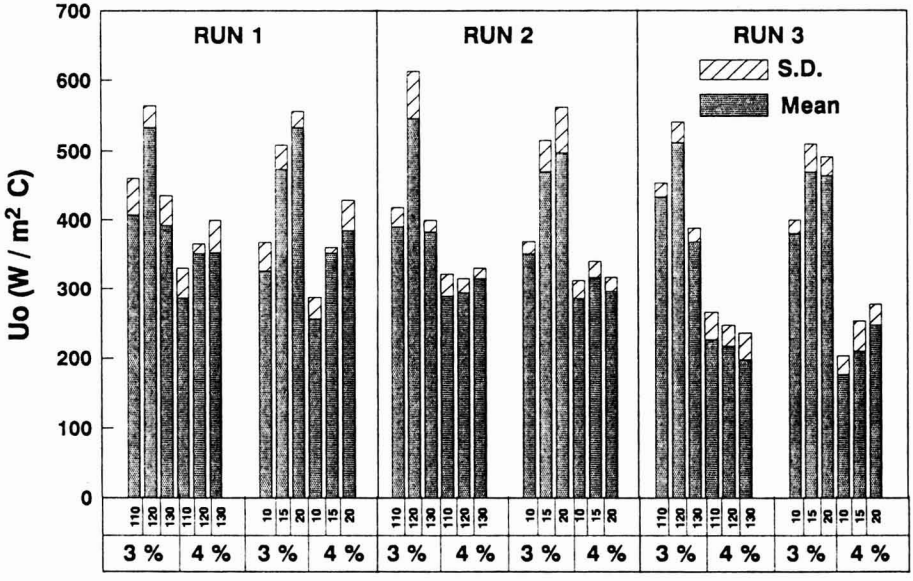

FIG. 7. OVERALL HEAT TRANSFER COEFFICIENT AS INFLUENCED BY TEMPERATURE (110, 120 AND 130C), ROTATION SPEED (10, 15 AND 20 RPM) AND STARCH CONCENTRATION (3% AND 4%) FOR ALL THREE RUNS

Dimensionless Correlations

The overall heat transfer coefficient Uo was related to rotation speed (N, rpm), retort temperature (T_r, C) and initial apparent viscosity (η_i, Pas) as shown below:

$$\log_e U_o = 5.99\text{-}2.72 \times 10^{-3} T_r + 3.76 \times 10^{-2} N - 3.55\ \eta_i \ (R^2=0.70). \quad (7)$$

Only apparent viscosity and speed of rotation were significant ($p<0.01$) as individual factors. The conventional dimensionless correlation of Nu with GRe and GPr (as employed by Deniston *et al.* 1992) gave a poor R^2 of 0.54. Some nonconventional correlations were then explored to improve the correlation. Since the speed of rotation had a significant influence on overall heat transfer coefficient, Froude number was employed as a dimensionless speed of rotation. The second major factor was viscosity, which was taken in the dimensionless form as specific apparent viscosity (η_{sp}).

The logarithm of the Nusselt number was correlated with Froude number and specific apparent viscosity as shown in Eq. (8):

$$\log_e Nu = 4.604 + 152.26\ Fr - 0.000329\ \eta_{sp} \quad [R^2 = 0.65] \quad (8)$$

Figure 8 is a response surface plot of Nusselt number as a function of Froude number and the apparent specific viscosity which demonstrates that the heat transfer coefficient decreased with the apparent specific viscosity and increased with rotation speed, the former being a more dominant factor.

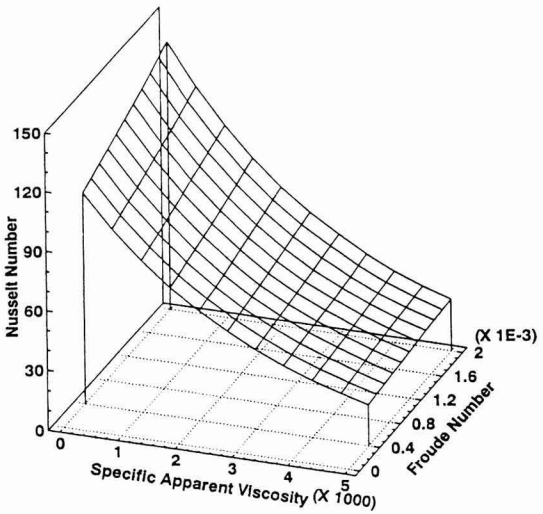

FIG. 8. RESPONSE SURFACE PLOT FOR NUSSELT NUMBER AS A FUNCTION OF SPECIFIC APPARENT VISCOSITY AND FROUDE NUMBER

CONCLUSIONS

Heating rates during rotation processing of 3% and 4% starch solutions were influenced ($p<0.05$) by starch concentration, retort temperature and rotation speed. Results obtained in this study indicate good correlations of cook value of processed starch with product apparent viscosity and color changes. Rotation speed and apparent viscosity of the samples were found to have the greatest influence on the heat transfer coefficient. As a result of this finding, the Nusselt number was correlated with Froude number and apparent specific viscosity.

ACKNOWLEDGMENTS

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NOMENCLATURE

A	=	Total external surface area of can (m2)
C_o	=	Cook value (min)
C_p	=	Product specific heat (J/kg K)
D_r	=	Diameter of rotation (m)
f_h	=	Heating rate index (min)
F_o	=	Process lethality (min)
F_r	=	Rotational Froude Number ($D_r N^2/g$)
g	=	Gravitational force (m/s^2)
GPr	=	Generalized Prandtl number $[(C_p m\ 8^{n-1})\ \{(3n+1)/4n\}^n]\ /\ [k\ (N/60)^{1-n}]$
GRe	=	Generalized Reynolds number $\{D_r^2\ (N/60)^{2-n}\ \rho\ \}\ /\ [m\ 8^{n-1}\ \{(3n+1)/(4n)\}^n]$
h	=	Surface heat transfer coefficient (W/m^2C)
j_{ch}	=	lag factor
k	=	Product thermal conductivity (W/mC)
m	=	Consistency coefficient (Pas^n)
m_p	=	Product mass (kg)
N	=	Rotation speed (rpm)
n	=	Flow behavior index (dimensionless)
Nu	=	Nusselt number (U_oDr/k)
σ	=	Shear stress (Pa)

T_p = Product Temperature (C)
T_r = Retort Temperature (C)
U_o = Overall heat transfer coefficient (W/m^2 C)
η_i = Initial apparent viscosity (Pas)
η_{sp} = Specific apparent viscosity = $[m8^{n-1}/(N/60)^{1-n}]\ [(3n+1)/(4n)]^n/\eta_{water}$
$\dot{\gamma}$ = Shear rate (s^{-1})
ρ = Product density (kg/m^3)

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PROPERTIES OF BATTERS AND STORAGE STABILITY OF FRANKFURTERS CONTAINING PREEMULSIFIED FAT STABILIZED WITH SOY PROTEINS[1]

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ABSTRACT

Soya proteins were incorporated into meat batters either as part of preemulsified fat (PEF) or as the powder. Batters made with PEF had higher viscosity, water holding capacity, adhesive properties, and improved batter stability. During storage the experimental frankfurters had similar microbial count, water activity and TBA values, but frankfurters containing soya proteins added as PEF had lower total volatile nitrogen than those samples with soya protein added as the powder. Samples containing PEF during 30 days of storage were less bitter and beany than those with powdered proteins. Incorporation of soya proteins in the PEF form improved functionality of sausage batters, and therefore, could increase the usage of soya proteins in comminuted meats.

INTRODUCTION

Interest in the use of vegetable proteins as additives in food products especially comminuted meat products, has increased markedly in recent years. Soya flours, concentrates, and isolates have been used extensively in prepared foods in earlier years but soya off-flavors (beany and bitter characteristics) have significantly limited their usage. Two crucial factors affecting acceptability of protein additives (soya proteins) in sausage products are storage stability

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(chemical and microbial), and functional properties.

Incorporation of soya proteins in comminuted meat products affects the functional performance of the system. Numerous experiments have been conducted since the mid 1970s and results have showed that use of soya ingredients in comminuted meat products resulted in substantial reduction in cooking shrinkage and higher yields (Williams and Zabik 1975; Ziprin *et al.* 1981; Ali *et al.* 1982; Rice *et al.* 1989).

Many researchers have studied the effect of the functionality of soya proteins in frankfurters, but no research had been done on soya protein incorporated as preemulsified fat (PEF) in sausage products. Ultrasonic emulsification was utilized to prepare PEF from melted fat, water, and different protein additives (Zayas 1985). This study showed that the frankfurters containing PEF had improved functional properties. Also, incorporation of PEF in frankfurters using corn germ protein as the stabilizer in PEF significantly increased the water-holding capacity (WHC) and yield of the finished product (Lin and Zayas 1987).

Besides functional properties, storage stability is also important in comminuted meats. Lipid oxidation of comminuted meat products is one of the major processes responsible for the deterioration of quality. Most frankfurters contain fairly high levels of fat and, therefore, are susceptible to deterioration during storage. Soya proteins contain some substances with antioxidant properties in food systems (Rhee *et al.* 1979; Pratt *et al.* 1981). Berry (1990) reported that negative effects due to frozen storage such as changes in color, flavor, odor and fat oxidation could be reduced by addition of soy proteins.

Romijn *et al.* (1991) observed a proportionate decrease in TBA values by the addition of 8, 16, and 24% soya isolate to a cooked, refrigerated, beef system. Cunningham and Bowers (1977) combined meat with 10% textured soya protein: the soy patties had lower TBA values than the 100% meat sample. Total aerobic plate counts were similar for both samples. However, Craven and Mercuri (1977) reported higher total aerobic plate counts after 10 days storage (4C) in patties containing textured soya protein. Draughon *et al.* (1982) reported similar results. Thompson *et al.* (1984) showed that soya isolate emulsions had higher microbial counts than emulsions containing vegetable protein flour or soya concentrate. However, no effect of storage time on TBA values was demonstrated.

The purpose of this study was to (1) investigate the effect of soya proteins incorporated in the preemulsified form on the functional characteristics of raw sausage batters and (2) to evaluate frankfurters at 15 day increments during 45 days of storage for stability (sensory, chemical, and microbial).

MATERIALS AND METHODS

Sample Preparation

Seven different frankfurter treatments were prepared in triplicate with beef (Kansas State University Meat Lab). The flow chart for the production of the frankfurters is presented in Fig. 1. Fresh beef (blends of lean cuts with about 12% fat), was ground (9.38 mm plate), mixed thoroughly, and reground through a 4.69 mm plate. The samples were randomly divided into blocks 4.5 kg, sealed in vacuum packages and stored at −12C. Frozen meat was thawed for 24 h, reground through a 4.69 mm plate, mixed (Hobart bowl mixer) with 2% table salt, and half the total added ice water, then 0.25% Prague powder (containing 6.25% sodium nitrite, Griffith Lab, Alsip, IL), and 1% sugar as related to the weight of meat. Next, the soya proteins were added. Treatments included either soya flour (SF, 3.5%), soya concentrate (SC, 3.5%) (both acquired from Central Soya, Ft. Wayne, IN), or soya isolate (SI, 2%) (Protein Technology International, St. Louis, MO) or no soya added (control). From each experimental sample containing soya protein, one was prepared with soya as powder (p), and the other with soya protein incorporated in PEF (e).

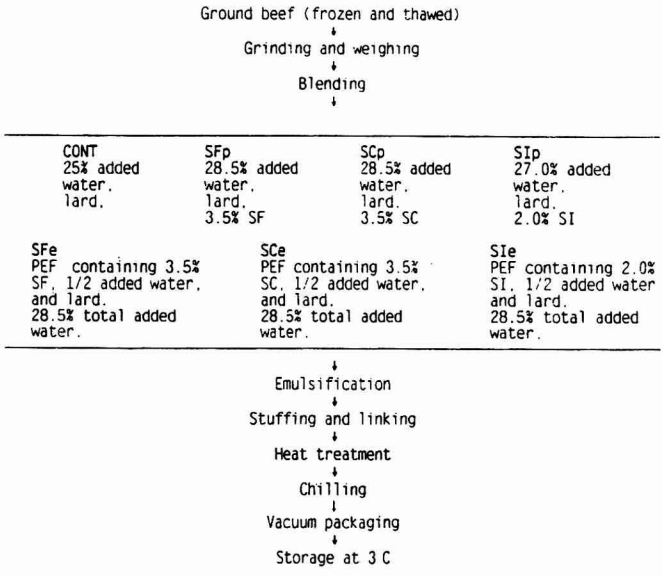

FIG. 1. THE FLOW CHART OF FRANKFURTER PRODUCTION
CONT = control, SFp = soya flour added as powder, SCp = soya concentrate added as powder, SIp = soya isolate added as powder, SFe = soya flour added as PEF, SCe = soya concentrate added as PEF, SIe = soya isolate added as PEF. Also, SF, SC, SI, represent soya flour, soya concentrate and soya isolate. PEF = Preemulsified fat.

After addition of soya proteins, as powder or as PEF, unemulsified lard was added to the control and the powdered soya samples, and then the last half of the total ice water was added to all samples. The level of added lard was in the range to maintain 22% fat content in finished product. Lard contained BHA and citric acid (Armour Co., Omaha, NE). Mixing continued for 5 min. The seven sausage batters were comminuted in an emulsifier (Griffith Design and Equipment., Chicago, IL), having a 1.7 mm plate. The final temperature of the batters was kept below 15C. Samples of each treatment were used for batter analyses.

After the meat batters were emulsified and stored in a freezer for 10 min, the 7 batters were stuffed into 24 mm casings with a vacuum stuffer (Vemag Co., Robot 500, Model 128). The links were formed 10 cm in length and hung on a cooking rack and cooked in a Maurer smokehouse. The cooking schedule followed was; 48C for 10 min, 55C for 30 min, smoking for 5 min at 55C, and cooking at 80C to an internal temperature of 70C. Temperatures were measured using thermocouples and a Doric Minitrend Data Logger (Model 205B-1-C-OTC). The frankfurters were chilled by a water shower, peeled, vacuum packaged (Super-Vac, Smith Equipment Co., Clifton, NJ), and stored in a refrigerator at 3C.

The three PEF samples were made by blending a mixture of soya protein (2.0% SI, or 3.5% SF or SC of the formulation weight) and water (1/2 the added water of the formulation) in a Oster blender (Model 548-41A) at 10,000 rpm for 2 min. The protein slurry then was incubated for 30 min in a Gallenkamp water bath (Model 394475) at 80C. Melted lard was added slowly by drops and emulsified with the stabilizer solution for 5 min. The PEF was made 2 h prior to addition to the batter.

The control frankfurter (no soya protein added) contained 25% added water (meat weight). In order to maintain a proper balance between protein, fat, and water, higher levels of water were added to the experimental samples (although we realized that the ionic strength would change). The experimental samples were formulated from the control sample with 1% extra added water for every 1% soya protein product added in the formulation. Therefore, the two experimental SF samples and two experimental SC samples contained 28.5% added water, whereas the experimental samples containing SI had 27.0% added water.

Batter Analyses

Sausage Batter Stability. Batter stability was determined by a procedure similar to that of Townsend *et al.* (1968). Clear 50 ml, plastic centrifuge tubes (27.5 ml × 110 ml) were filled with 23.3 g of raw sausage batter and centrifuged at low speed for 5 min to evacuate air that might have been trapped

during hand stuffing (Whiting, 1984). The tubes were covered to eliminate water evaporation during heating. The emulsions were heated in a water bath (Gallenkamp, model 394475) at 85C for 30 min. The plastic centrifuge tubes (24) were used for each treatment. Three tubes filled with batter from the same treatment were inverted into a glass funnel containing a metal screen filter. The cook out material was collected into graduated cylinders for measuring the volume of fluid (water), fat, and solids/ 70 g sample.

Water Holding Capacity (WHC). The WHC was determined by the technique of Hamm (1972). A sample of batter, approximately 0.3g, was weighed and placed on Whatman #1 filter paper, which was placed between two plexiglass plates and pressed by 1 kg wt for 20 min. The areas of separated water and meat pressed were measured with a compensating polar planimeter.

Viscosity. The viscosity of the batter after 1 h incubation at 3–4C was measured with a RV8 viscometer (Viscometers UK Ltd., London, England) with a digital system. Spindle # 7 was used with a speed of 10 rpm. Five readings for each batter were taken for statistical analysis. All readings were recorded in centipoise (cps).

Adhesiveness. The adhesiveness was measured on an Instron Universal Testing Machine (Model 1122 Instron Corp., Canton, MA). A 200 g sample of batter (about 2 cm deep) filled the bottom of a round metal extrusion cell (model# A312-13, 11.0 cm × 10.6 cm). As the plunger was driven into the sample, at a crosshead speed of 10 mm/min, the product was compressed 0.1 cm. The plunger was then extracted immediately, and the force (kg) required to rupture the sample was recorded on chart paper moving at 50 mm/min.

Storaqe Stability

Water Activity (a_w). The a_w was measured at day 1, 15, 30, and 45 with a thermoconstanter (Model HTCI-2, Beckman Industrial Co., Cedar Grove, NJ). Samples were finely ground prior to each determination and four replications were performed on each treatment.

TBA Test. The TBA test was used to study fat oxidation during storage for 45 days at 15-day increments using a modification procedure of Witte *et al.* (1970). A 10 g sample was extracted with 40 ml of 9% perchloric acid and filtered through Whatman #2 filter paper. Seven 5-ml aliquots from the filtrate of each sample were added to 5 ml of 0.02M TBA solution, incubated for 17 h at ambient temperature, and measured for malonaldehyde by a spectrophotometer (Coleman Model 1240, Perkin-Elmer Co., Maxwood, IL) at

530 nm. A standard curve was produced by preparing a solution of 1.1.3.3-tetraethoxypropane (TEP) at different concentrations and adding the solutions to 0.02 M TBA for 17 h and observing the absorbance at 530 nm also. The TBA values were reported as mg malonaldehyde/kg sample.

Total Volatile Nitrogen (TVN). Total volatile nitrogen was determined by a method similar to the distillation technique of Cobb *et al.* (1973). A 10 g sample was added to 40 ml of 7% trichloracetic acid for extraction and blended for 2 min. The solution was filtered through Whatman #2 filter paper. Seven 5-ml aliquots were added to 5 ml of saturated Na_3P0_4. A micro-Kjeldahl apparatus was used for the rapid TVN analysis. The volatile nitrogen was trapped in 5 ml of boric acid containing Tashiro's indicator. Distillation was continued until approximately 10 ml of distillate was collected. TVN was calculated as mg N/lOOg sample at 1, 15, 30, and 45 days of storage at 4C.

Microbial Analysis. The experimental (except containing SI) and control samples were prepared for microbial tests from each frankfurter at 1, 15, 30, and 45 days of storage by homogenizing 10 g of sample with 90 ml sterile diluent in a Stomacher (Lab Blender STO-400, Tekmar, Co., Cincinnati, OH) for 1 min. Serial dilutions were prepared by transferring the appropriate 10 ml aliquot to 90 ml sterile buffer. Aerobic plate counts were made by inoculating duplicate prepoured plates of standard plate count agar (Difco Laboratories, Detroit, MI) using aseptic technique, with 0.1 ml or 1 ml inoculum from the serial dilutions. The psychrophilic aerobic plate counts were determined after incubation at 6C for 10 days (APHA 1984).

Sensory Evaluations

A trained 7-member sensory panel was used to evaluate the frankfurters at days 1, 15, and 30. Aroma characteristics evaluated were meaty, soya, and aroma acceptability. Flavor notes evaluated were meaty, soya (beany), bitter, and acceptability.

Six frankfurters were randomly selected from each treatment, cooked in boiling water for 2 min and cut into 2 cm-long sections. These sections were served to panelists in warmed glass custard cups covered with a watch glass. The references selected include: beef bouillon solution, frankfurters containing 15% SF, coffee, and SF and SC that had been stored for 4 years in order to develop a strong beany reference. The panelists were trained in four sessions to be familiar with the references and were trained to recognize different concentrations of soya proteins added to frankfurters (from 2 to 10%). Panelist first evaluated aroma then flavor of the frankfurters. Distilled water, apples, and crackers with salt free tops were provided to each panelist to eat between

samples to clear their palates of residual flavor. An unstructured intensity linear scale with 60 points (60 = strong, O = none or weak) was used for the evaluation.

Statistical Analyses

The frankfurters were produced at 3 different times (trials). The experiment was a randomized block design with meat or trials as the block effect. All treatments of the experiment appeared in each block. Data were analyzed by analysis of variance using the General Linear Model (GLM) procedure (SAS 1985). The block × treatment interaction was used as the error term to eliminate its effects. When F values were significant in the analysis of variance, the least significant difference (LSD) was calculated to test differences among treatments. Two-way analysis of variance was used to analyze the effect of storage time and treatment. If significant interactions occurred between treatment × storage time, then least square means were used to discriminate differences between samples.

RESULTS AND DISCUSSION

Batter Analyses

Batter Stability. Figure 2 represents the stability of the batters containing soya proteins and the control. Batter stability is used to determine the fat and water binding ability of the batter and is very crucial to frankfurter production. SIe displayed the least liquid and fat separation, followed by SCe. Other researchers have also reported that SI had superior emulsion stabilizing properties compared to SC and SF (Lauck 1975; Parks and Carpenter 1987). The six experimental batters lost significantly ($P < 0.05$) less water and fat than the control emulsions. Rice *et al.* (1989) also reported similar findings for ground beef patties. All soya protein samples incorporated as PEF (SFe, SCe, and SIe) retained more moisture than their counterpart samples with soya protein added as the powder (SFp, SCp, and SIp). The control and SFp samples released the most fat. The samples containing soya as PEF were superior to the samples containing soya protein added as powder since less fat was released, indicating an elevated stability as a result of preemulsification of the soya proteins. Increased stability of the batter also increased yield and decreased cook losses of the frankfurters (Lecomte *et al.* 1993). No differences existed between any of the treatments for solids released upon heating. Therefore, sausage batters formulated with soya proteins incorporated as PEF had significantly ($P < 0.05$) better emulsion stability than emulsions formulated with soya added as the powder, as exhibited by significantly lower cook loss (water) and ml fat released.

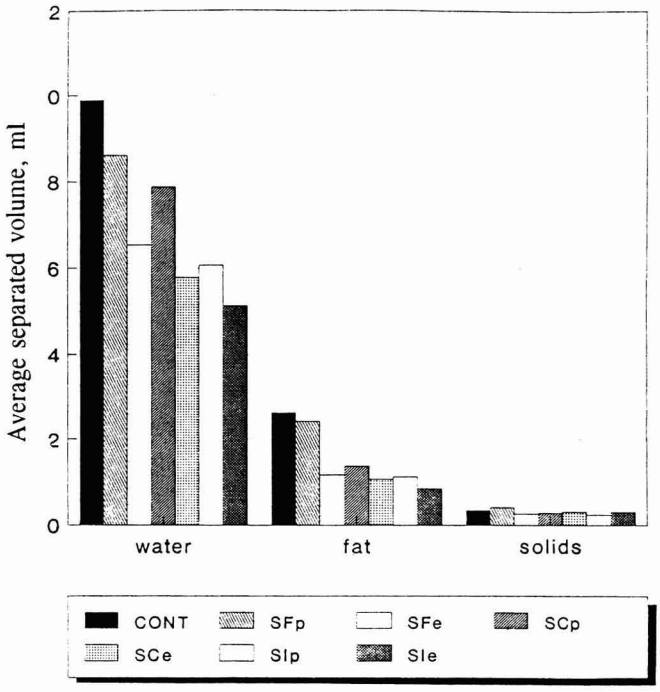

FIG. 2. THE EFFECT OF PREEMULSIFICATION OF SOYA PROTEINS ON SAUSAGE BATTER STABILITY

CONT = control, SFp = soya flour added as powder, SFe = soya flour added as preemulsified fat (PEF), SCp = soya concentrate added as powder, SCe = soya concentrate added as PEF, SIp = soya isolate added as powder, SIe = soya isolate added as PEF. LSD = 0.440, 0.322, and 0.009 for water, fat, and solids, respectively.

Water Holding Capacity. Mean values for WHC are presented in Table 1. The WHC was significantly influenced by the incorporation of soya proteins as PEF. All three batters containing PEF (SFe, SCe, and SIe) showed superior WHC compared to soya proteins added as powder (SFp, SCp, and SIp). Also, the control sample displayed the poorest water-holding properties (0.559). The SF and SC samples had the highest amount of added water (28.5% of the formulation) and were equal in water-holding with the SI sample. Therefore, the protein and carbohydrate portions of the SF and SC may help bind the extra water added. Hence, the binding properties of soya samples added as PEF were enhanced over the samples with soya added as powder. Lin and Zayas (1987)

reported the WHC also was improved by preemulsification of plant proteins (corn germ protein). Improving the WHC and batter stability (Fig. 2) through the addition of soya proteins as PEF resulted in a final product with improved yields.

TABLE 1.
WATER-HOLDING CAPACITY (WHC), VISCOSITY, AND ADHESIVENESS OF RAW SAUSAGE BATTERS

Sample[1,2]	WHC	Viscosity(cPs)	Adhesiveness(kg)
Control	.55a	325,000a	0.85a
SFp	.64b	348,000bc	1.01b
SFe	.74c	367,550de	1.14d
SCp	.63b	346,330b	0.98b
SCe	.76c	359,254cd	1.05c
SIp	.66b	360,760d	1.18e
SIe	.75c	376,550e	1.17e
LSD	.042	11,670	0.022

[abcde]Means followed by different letters in the same column are significantly different at $P < 0.05$.

[1]Means from 8 replications.

[2]SFp = soya flour added as the powder, SFe = soya flour added as preemulsified fat (PEF), SCp = soya concentrate added as powder SCe = soya concentrate added as PEF, SIp = soya isolate added as powder, SIe = soya isolate added as PEF

Viscosity. The mean values for the viscosity are presented in Table 1. The control sample was least viscous (325,000 cPs). SIe and SFe showed the highest viscosity ($P < 0.05$), 376,000 and 367,550 cps, respectively. All sausage batters containing soya added as PEF (SFe, SCe, and SIe) had higher viscosity readings than those containing the powder forms of the soya protein (SFp, SCp, SIp). Preemulsification and incorporation of PEF improved the binding ability of the proteins and binding of the batter particles and, hence, increased viscosity.

Adhesiveness. The SIe and SIp samples showed the highest adhesive properties, whereas the control (no soya protein additive) was least adhesive. The SFe and SCe batters had higher adhesiveness values than their counterparts, the SFp and SCp batters. Higher adhesive properties of sausage batters reflected favorable stability from improved binding of water, fat and protein particles.

Storage Stability

Storage stability of the sausage products containing soya proteins and the control was tested by water activity (a_w), chemical analyses (TBA and TVN tests), and microbial tests.

Water Activity. The water activity (a_w) is the amount of water available for microbial growth. From the F-values (data not shown), storage time and treatment strongly affected a_w. A significant interaction occurred between treatment and time of storage ($P < 0.05$) for a_w. Therefore, the influence of storage time was treatment dependent. The effect of preemulsification of soya proteins and storage time on water activity is provided in Fig. 3. Water activity significantly increased during the 45 day storage period, and increased more intensely between day 15 and 30. The control samples maintained the highest a_w after day 15 and SCe had the lowest value after 15 days of storage ($P<0.05$). The SCp sample showed higher a_w values than SCe at day 30, and 45. No significant differences existed between SFp and SFe, or SIp and SIe throughout the 45 day storage period.

TBA Test. TBA values were used as an index for fat oxidative stability during storage of the frankfurters. There was a significant interaction between time of storage and treatment ($P < 0.05$). The effect of preemulsification and storage of frankfurters with soya proteins on TBA values is given in Fig. 4. The control sample containing no soya protein added had the highest TBA value at day 1, but the SCp sample had higher values at day 15, 30, and 45. Other research also has shown reduction in TBA values when soya proteins are incorporated in comminuted meat products (Cunningham and Bowers 1977).

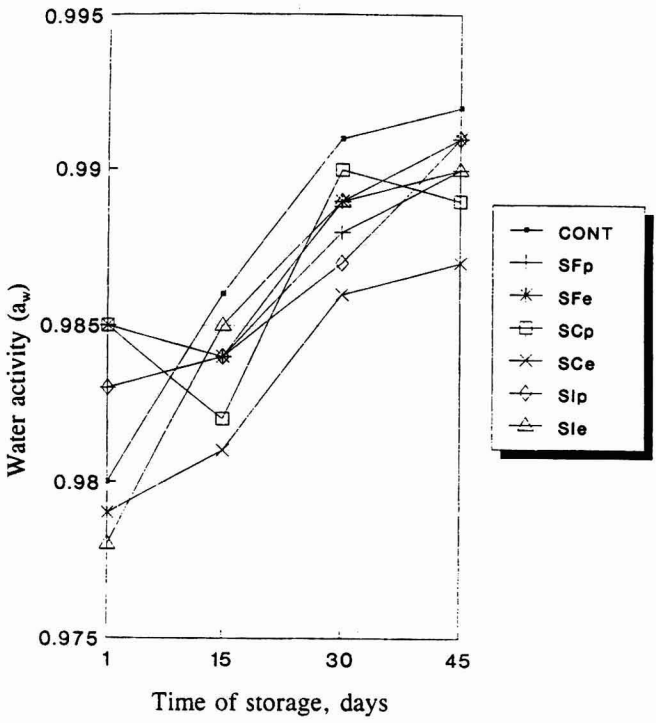

FIG. 3. THE EFFECT OF PREEMULSIFICATION AND STORAGE OF FRANKFURTERS WITH SOYA PROTEINS ON WATER ACTIVITY

CONT = control, SFp = soya flour added as powder, SFe = soya flour added as preemulsified fat (PEF), SCp = soya concentrate added as powder, SCe = soya concentrate added as PEF, SIp = soya isolate added as powder, SIe = soya isolate added as PEF.

This reduction in TBA values may be a direct effect of the antioxidative properties of the soya proteins. The SCe sample had significantly lower TBA values during all days of storage than the SCp sample. Generally, preemulsification of soya proteins seemed to have little effect on the TBA values during storage, except for SCe. There was a significant decrease in TBA values during the storage period (except SCp). A reduction in TBA values may be a result of malonaldehyde being unstable and further undergoing extensive modifications at advanced stages of oxidation. Since malonaldehyde is the primary TBA-reactive substance, at later stages of storage and oxidation less malonaldehyde is available to form the TBA chromagen responsible for absorbance at 530 nm. Also, malonaldehyde may react with protein during lipid oxidation, causing a reduction in TBA values (Melton 1983).

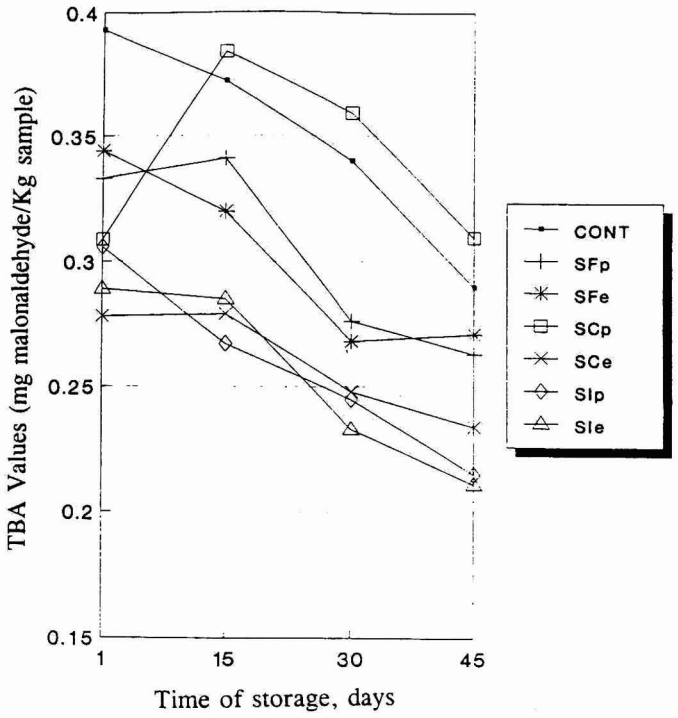

FIG. 4. THE EFFECT OF PREEMULSIFICATION AND STORAGE OF FRANKFURTERS WITH SOYA PROTEINS ON THIOBARBITURIC (TBA) VALUES
CONT = control, SFp = soya flour added as powder, SFe = soya flour added as preemulsified fat (PEF), SCp = soya concentrate added as powder, SCe = soya concentrate added as PEF, SIp = soya isolate added as powder, SIe = soya isolate added as PEF.

Total Volatile Nitrogen (TVN)

Total volatile nitrogen measures protein decomposition. There was a significant interaction between storage time and treatment effects ($P < 0.05$). Therefore, storage time and treatment factor affected TVN values. Fig. 5 represents the effect of storage time and preemulsification of soya proteins in frankfurters on the TVN. A sharp increase in TVN values was recorded for the first 15 days of storage. The rate of increase was reduced from day 15 to 30 and no increase was observed from 30 to 45 days storage. Overall, an increase in TVN occurred during the 45-day storage study. A low level of TVN increase was found for samples containing SIe and SIp. Also, SFe and SCe retained lower TVN values during the 45-day storage time than SFp and SCp (although no significant difference was observed at day 30 between SFp and SFe). Therefore, preemulsification may have a significant reduction effect on proteolytic

activity during storage. TVN represents changes of the nitrogenous compounds that may occur as a result of both endogenous enzymic (proteolytic) activity and the activity of specific bacteria.

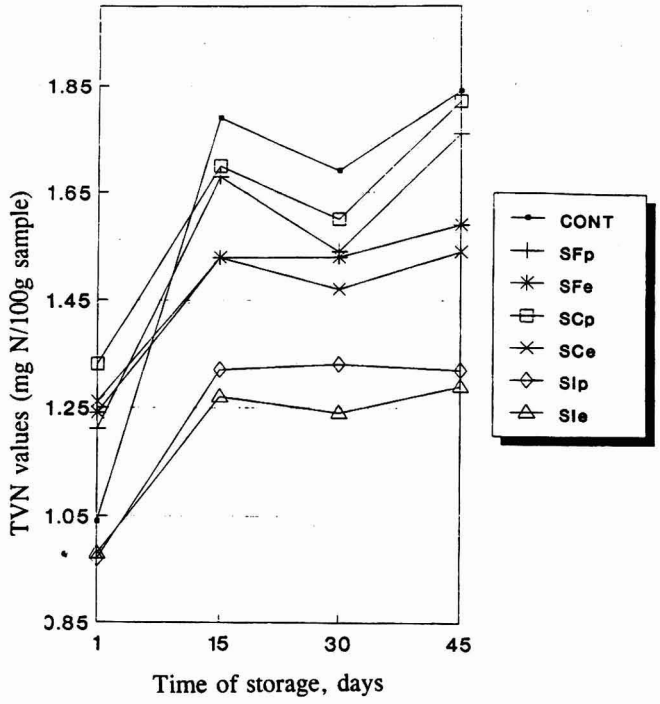

FIG. 5. THE EFFECT OF PREEMULSIFICATION AND STORAGE OF FRANKFURTERS ON TOTAL VOLATILE NITROGEN (TVN)

CONT = control, SFp = soya flour added as powder, SFe = soya flour added as preemulsified fat (PEF), SCp = soya concentrate added as powder, SCe = soya concentrate added as PEF, SIp = soya isolate added as powder, SIe = soya isolate added as PEF.

Microbial Test

The results of the total plate count (TPC) are presented in Table 2. The total number of psychrophiles increased from 1 to 15 and 30 through 45 days of storage for all 5 treatments. The TPC ranged from 1.68×10^2 to 2.91×10^2 for day 1 and 8.13×10^5 to 1.02×10^7 for day 45. No differences existed among the treatments for day 1, day 15, and day 30. The control (no soya protein added) had significantly lower ($P < 0.05$) TPC values than the four samples containing soya protein during day 45 of storage. The SCp sample had significantly ($P < 0.05$) greater TPC than all treatments at day 45. Craven and Mercuri (1977) also reported higher total aerobic plate counts for comminuted meat products containing soya protein. They suggested that soya proteins might contain some unidentified growth factor that enhances microbial growth. Therefore, preemulsification of soya proteins had neither a positive or a negative effect on microbial growth.

TABLE 2.
TOTAL PLATE COUNT OF FRANKFURTERS CONTAINING SOYA PROTEINS DURING 45 DAYS OF STORAGE

	Storage time, days			
Sample[1,2,3]	1	15	30	45
Control	1.68×10^2a	3.18×10^3a	5.08×10^3a	8.13×10^5b
SFp	2.91×10^2a	9.46×10^3a	1.60×10^5a	2.46×10^6c
SFe	2.65×10^2a	1.20×10^4a	3.64×10^5a	5.90×10^6c
SCp	2.91×10^2a	3.73×10^4a	2.81×10^5a	1.02×10^7d
SCe	2.77×10^2a	8.47×10^3a	1.70×10^5a	2.81×10^6c

abcde means followed by different letters in the same column and are significantly different at $P < 0.05$

[1] SFp = soya flour added as powder, SFe = soya flour added as preemulsified fat (PEF), SCp = soya concentrate added as powder, SCe = soya concentrate added as PEF,

[2] Means from 4 replications

[3] MSE = 2.7×10^{10}

Sensory Analysis

The effects of preemulsification and 30 day storage on bitter flavor of frankfurters is presented in Fig. 6. The control, SIe, and SFe were least bitter among the samples. SCp and SFp were significantly more bitter than other samples during 30 days of storage. The samples containing soya proteins added as PEF were less bitter than the samples containing soya proteins added as powder showing a masking effect of the preemulsification of soya proteins.

The beany flavor of frankfurters containing soya proteins as PEF and powder and during 30 days of storage is presented in Fig. 7. The control frankfurters were lowest in beaniness after 1 and 30 days of storage. However, there were minimal differences in beany flavor for SIe, SFe, SIp and the all-meat control frankfurters after 15 days of storage. The SCp and SFp samples had the highest numerical scores for beany flavor in the range of 30 to 45 on the 60-point scale. The samples containing soya proteins added as PEF (SFe and SCe) were less beany than the samples containing soya proteins as powder. The significant reduction of the beany flavor was a result of a masking effect by the preemulsification of the soya proteins.

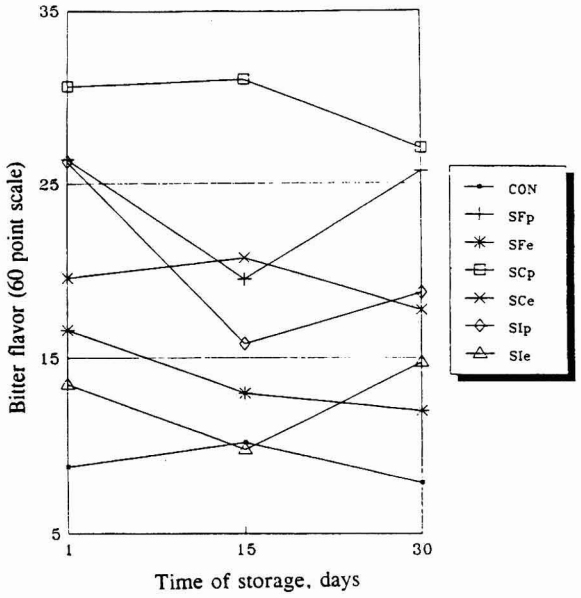

FIG. 6. THE EFFECT OF PREEMULSIFICATION AND STORAGE OF FRANKFURTERS WITH SOYA PROTEINS ON BITTER FLAVOR

CONT = control, SFp = soya flour added as powder, SFe = soya flour added as preemulsified fat (PEF), SCp = soya concentrate added as powder, SCe = soya concentrate added as PEF, SIp = soya isolate added as powder, SIe = soya isolate added as PEF.

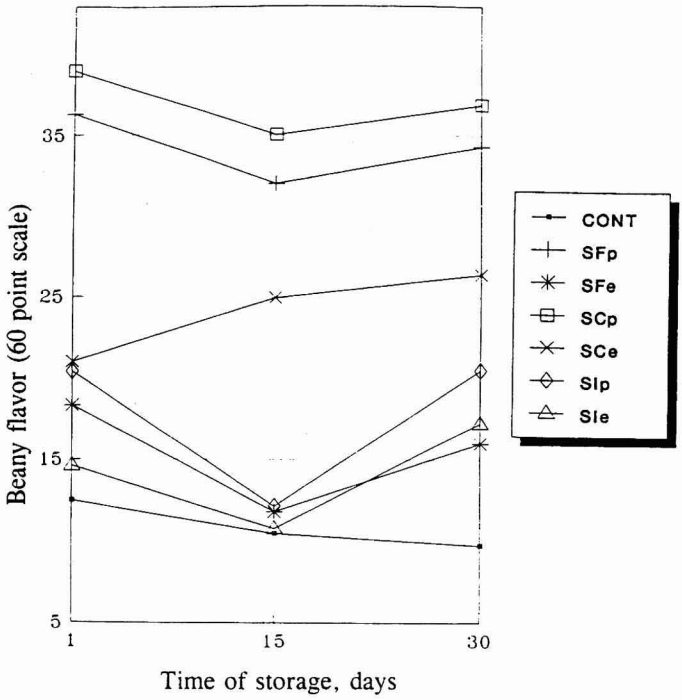

FIG. 7. THE EFFECT OF PREEMULSIFICATION AND STORAGE OF FRANKFURTERS WITH SOYA PROTEINS ON BEANY FLAVOR

CONT = control, SFp = soya flour added as powder, SFe = soya flour added as preemulsified fat (PEF), SCp = soya concentrate added as powder, SCe = soya concentrate added as PEF, SIp = soya isolate added as powder, SIe = soya isolate added as PEF

CONCLUSIONS

Soya proteins incorporated as a preemulsified fat in a comminuted meat product were shown to improve the viscosity, water holding capacity, adhesiveness and batter stability. During a storage period, samples with PEF had the same microbial, a_w, and TBA values, but had lower TVN values than samples containing soya as powder. Samples containing soya as PEF had lower bitter and beany flavor than the samples containing the powdered forms during the 30 day storage period. However, control had less bitterness and beany flavor than all soya treatments. These findings are important because soya proteins added as PEF improved the flavor quality and had enhanced functional

properties in frankfurters. These improvements in functionality and sensory quality characteristics can increase the usage of soya proteins as an ingredient in comminuted meats.

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EFFECTS OF TEMPERATURE AND SOLUTE ON THE MINIMUM WATER ACTIVITY FOR GROWTH AND TEMPERATURE CHARACTERISTIC OF SELECTED MESOPHILES AND PSYCHROTROPHS[1]

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ABSTRACT

The temperature effect on the temperature characteristic (energy of activation) and the minimum water activity (a_w) for the growth of the psychrotrophs P. fluorescens *and* B. thermosphacta *and the mesophiles* Salmonella typhimurium, Streptococcus faecalis *and* Staphylococcus aureus *were analyzed in liquid media. Experimental data show that the minimum a_w of mesophiles is higher than that of psychrotrophs and more sensitive to temperature changes. The minimum growth a_w is affected by the a_w-controlling solute. The temperature characteristic of psychrotrophs was significantly lower than that of mesophiles and suggests a strong synergistic effect of low temperature and reduced a_w on the growth of mesophiles. The effect of a_w on the temperature characteristic depends on the a_w-controlling solute.*

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INTRODUCTION

An emerging type of products are the so-called extended shelf-life refrigerated (ESLR) foods (Labuza and Breene 1989; Labuza *et al.* 1992; Li and Torres 1993b). In particular, the development of refrigerated intermediate moisture foods (RIMFs) combining the effects of refrigeration temperature and reduced water activity (a_w) is receiving increased attention (Corlett 1988; El-Hag 1988; Mory 1988; Simpson *et al.* 1989; Almonacid-Merino and Torres 1991a,b, 1993; Almonacid-Merino *et al.* 1993a,b). The a_w of RIMFs is in the 0.90–0.95 range (Farber 1985) and products with this a_w could be affected by short temperature abuse periods. The stability analysis of these products includes microbial challenge studies with a spoilage indicator selected on the basis of a_w and potential temperature abuse considerations.

Early work by Scott (1953) suggested a biological response to a_w largely independent of the chemical nature of the solute and the moisture content of the growth medium. However, later studies have shown an effect of the nature of the solute molecule on the microbial growth response. Calhoun and Frazier (1966) compared the effects of glucose and NaCl (at equivalent a_w) on the growth of *Escherichia coli, Pseudomonas fluorescens* and *Staphylococcus aureus*. These two solutes had the same effect on the growth of *S. aureus*, but NaCl inhibited the growth of *P. fluorescens* more than glucose. Marshall *et al.* (1971) compared the inhibitory effects of NaCl and glycerol on 16 strains of bacteria. Only three species responded identically to both solutes. When compared at similar a_w levels, glycerol was more inhibitory than NaCl to relatively salt-tolerant bacteria and less inhibitory than NaCl to salt-sensitive species.

In general, solutes can be divided into two groups. The first group includes solutes that readily permeate the cell and do not elicit an osmoregulatory response (Measures 1975; Sperber 1983). These compatible solutes include glycerol, glutamate (Measures 1975), sorbitol (Prior *et al.* 1987), glucose and arabinose (Roller and Anagnostopoulos, 1982). Another group is solutes incapable of penetrating a cell in large amounts, or more strictly defined, those whose uptake inhibits saturation kinetics, causes plasmolysis and induces an osmoregulatory response. The latter group includes most salts and sugars (Gould and Measures 1977) and in general they cause a more significant extension of microbial growth lag phase (Li and Torres 1993a). The characteristics of a solute combination affects the microbial growth response and thus the formulation of intermediate moisture foods (IMFs) requires experimental work to determine their microbial stability.

In refrigerated foods the most important spoilage microorganisms are psychrotrophic bacteria. Although molds and yeasts are more tolerant of a reduced a_w than these bacteria, the addition of fungistatic substances inhibits their growth. In refrigerated meat and processed meat products, *Pseudomonas*

fluorescens and *Brochothrix thermosphacta* are two microorganisms commonly associated with their spoilage. Under aerobic conditions and chill temperatures (2–20C) pseudomonas grow faster than other species, become predominant in the spoilage microflora with *P. fluorescens* often found in this mixed population (Gill and Newton 1977, 1980). The minimum a_w for the growth of this bacterium is around 0.934–0.954, depending upon the a_w-controlling solute (Li and Torres 1993a). During temperature abuse, mesophiles may proliferate and dominate the spoilage flora of RIMFs and could include *Staphylococcus aureus*, *Salmonella typhimurium* and *Streptococcus faecalis*. Although *S. aureus* is normally not a good competitor in high moisture foods containing a mixed flora, it can be in a low a_w environment where the growth of most competitive microorganisms has been restricted (Lee *et al.* 1981). This bacterium can usually grow down to $a_w \approx 0.83–0.84$ and produces an enterotoxin down to $a_w \approx 0.86$ (Tatini 1973; Troller 1973) but does not grow below 6.5C (Inger 1983).

Salmonella species have been the subject of much research because of their pathogenicity. The most common food vehicles of *Salmonella* species, i.e., egg, poultry, meat and meat byproducts (Jay 1978), are often ingredients of IMFs (Haas and Herman 1978). Although the a_w of an IMF does not permit *Salmonella* multiplication, it may allow cells to survive for an extended period (Goepfert *et al.* 1968).

S. faecalis grows well at a_w above 0.94 (Haas and Herman 1978) and thus could grow in RIMFs when temperature abused. Since *S. faecalis* survives low a_w environments better than *E. coli*, some food microbiologists suggest the use of enterococci rather than coliforms as an index of sanitation (Uzelar and Stille 1977).

Temperature abuse is a main concern of the refrigerated food industry (Corlett 1988; Mory 1988). Temperature increases accelerate the proliferation of the spoilage microflora (Jay 1978; Malcata 1990; Almonacid-Merino and Torres 1993; Almonacid-Merino *et al.* 1993a,b). Unfortunately, little information is available on the minimum a_w for microbial growth in the 2–20C range and thus it is difficult to select an a_w value (critical a_w) such that shelf-life remains acceptable even after temperature abuse. The shelf-life reduction caused by temperature abuse depends on the temperature characteristic of the potential spoilage bacteria or the pathogen compromising food safety. The term temperature characteristic represents the microbial growth activation energy derived from the Arrhenius equation and is a useful parameter to describe the growth-temperature relationship of most bacteria (Reichardt and Morita 1982; Stannard *et al.* 1985).

This study examines the effect of temperature (4–26C) on the critical a_w for the growth of *P. fluorescens*, *B. thermosphacta*, *S. aureus*, *S. typhimurium* and *S. faecalis* in liquid media with a_w controlled by NaCl, glycerol or sucrose. The temperature characteristics of these bacteria were also calculated to help

formulate RIMFs, and combined with mathematical models (Almonacid-Merino and Torres 1993; Almonacid-Merino *et al.* 1993a,b) could be used to estimate temperature abuse effects on the microbial shelf-life of RIMFs.

MATERIALS AND METHODS

Cultures

Brochothrix thermosphacta (ATCC 12706), *Pseudomonas fluorescens* (ATCC 17400), *Streptococcus faecalis* (ATCC 7080), *Salmonella typhimurium* (ATCC 13311) and *Staphylococcus aureus* (ATCC 13566) were obtained from the American Type Culture Collection (Rockville, MD). The inoculum for the growth studies were prepared by transferring a loopful of culture from a nutrient broth or brain heart infusion slant to a flask with the corresponding broth (Difco, Detroit, MI). Brain heart infusion (BHI, pH 7.4 $\pm$ 0.2 at 25C) was the medium for *B. thermosphacta* and *S. faecalis*. Nutrient broth (pH 6.8 $\pm$ 0.2 at 25C) was used for *S. typhimurium*, *S. aureus* and *P. fluorescens*. Media were autoclaved for 15 min at 121C (15 psi).

Measurement and Adjustment of a_w

The a_w of media was adjusted using glycerol, sodium chloride or sucrose. After sterilization, the a_w was measured at the incubation temperature using a Hygroline sensor assembly (Model EBS, Beckman Industrial Inc., Cedar Grove, NJ) attached to an electric hygrometer recorder (Model VFB2, Beckman Industrial, Inc.). The reproducibility of this instrument is $\pm$ 0.1%, (Beckman Industrial, Inc.). Experimental data is reported with a 0.001 a_w units precision and the a_w meter was recalibrated on a weekly basis. In the case of a_w conditions leading to long lag phase ($>$ 5 days) and slow growth rate, the a_w was also measured at the end of the experiment.

Growth Studies

Erlenmeyer side-arm flasks containing 30 ml medium were inoculated with cultures (0.1 ml/flask) in the mid exponential phase of growth (10^8 CFU/ml). The flasks were incubated at controlled temperatures ($\pm$0.5C) for up to 15 days on an orbital shaker (120 rpm). An apparent specific growth rate was determined by measuring with a Spectronic 20 (Bausch and Lomb, Rochester, NY) the optical density (OD) of the growth medium at 600 nm (Li and Torres 1993a).

RESULTS AND DISCUSSION

Temperature Effect on the Minimum a_w for Growth

Linear correlations of the minimum a_w as a function of temperature for the growth of all test microorganisms examined in this study show significant solute effect on the minimum a_w for growth (Fig. 1). A visual inspection of the slopes of the minimum a_w for the growth of the mesophiles and psychrotrophs tested show major differences. This observation suggests an osmoregulatory mechanism (Gould and Measures 1977) for mesophiles more sensitive to temperature than that for psychrotrophs. At refrigeration temperature the minimum a_w for the growth of mesophiles is higher than that of psychrotrophs. Psychrotrophs can adjust the fatty acid composition of the cell membrane to retain its fluidity when temperature is lowered (Joyce *et al.* 1970; Gill 1975). The temperature-effect difference might also be related to the inability of mesophiles to synthesize certain proteins at low temperature (Broeze *et al.* 1978).

The minimum a_w for *B. thermosphacta* was lower than that for *P. fluorescens* when glycerol or NaCl were used to reduce a_w (Fig. 1 a,b) but were similar when sucrose was used (Fig. 1c). The minimum a_w for the growth of *S. aureus* in media with NaCl decreased from 0.926 to 0.873 when the temperature changes from 26 to 12C, indicating a large synergistic effect of refrigeration temperature and reduced a_w. Similar trends are observed for *S. faecalis* and *S. typhimurium*.

The minimum a_w for the growth of psychrotrophs was only slightly affected by temperature. However, other growth parameters such as maximum cell density (OD_{600}), specific growth rate and lag time were affected by temperature. *P. fluorescens* was incubated at 4, 8, 16, and 26C in media with 25% (a_w = 0.947) and 27.5% (a_w = 0.943) glycerol. At a_w = 0.947 and 26C (Fig. 2a), the growth of *P. fluorescens* reached only 0.02 OD_{600} units. At 8 and 4C, the maximum growth density reached only 0.06 and 0.05 OD_{600} units (ca. 8.0 and 6.0 $\times$ 10^7 CFU/ml, respectively), which is 1/20 of the growth observed in nutrient broth without added glycerol. At a_w = 0.943, no growth was detected at 26C and only slight growth was observed at 16, 8 and 4C (Figure 2b).

Application of the Arrhenius Model

Specific growth rate in media with a_w controlled by NaCl, glycerol or sucrose and incubated at 4–26C was used to construct Arrhenius plots for the growth of *B. thermosphacta*, *P. fluorescens*, *S. faecalis*, *S. typhimurium* and *S. aureus*. Examples of these curves are shown in Fig. 3. This required interpolation of specific growth rate values because media with the same solute

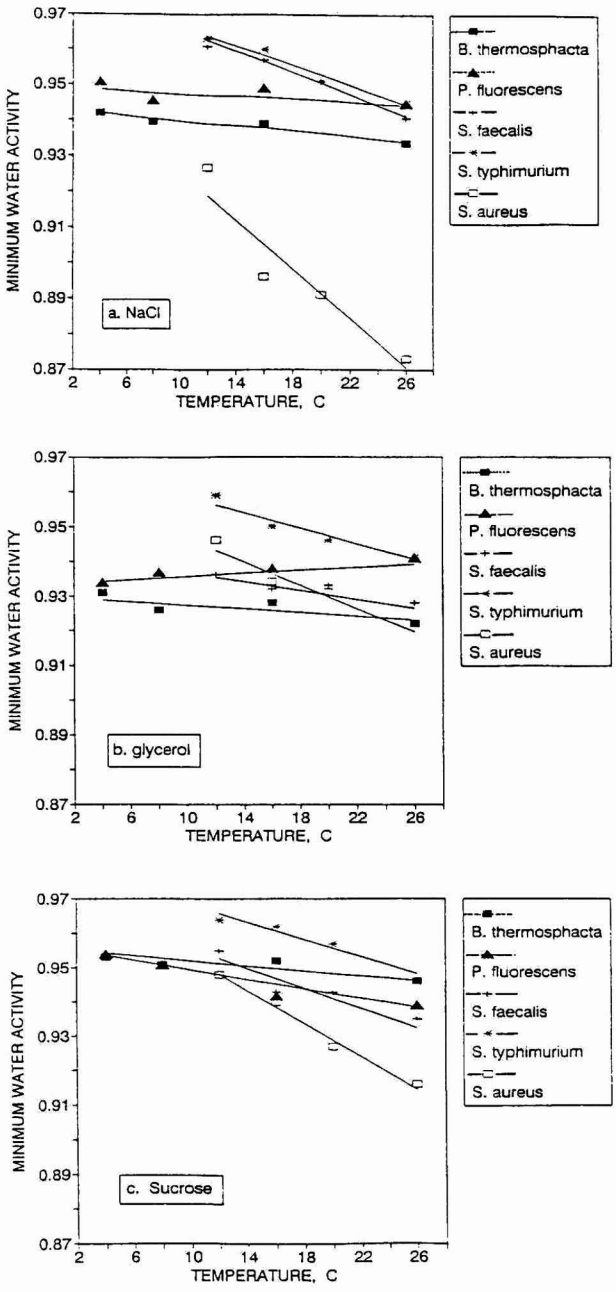


FIG. 1. EFFECT OF TEMPERATURE ON THE MINIMUM a_w FOR GROWTH IN LIQUID MEDIA WITH REDUCED a_w

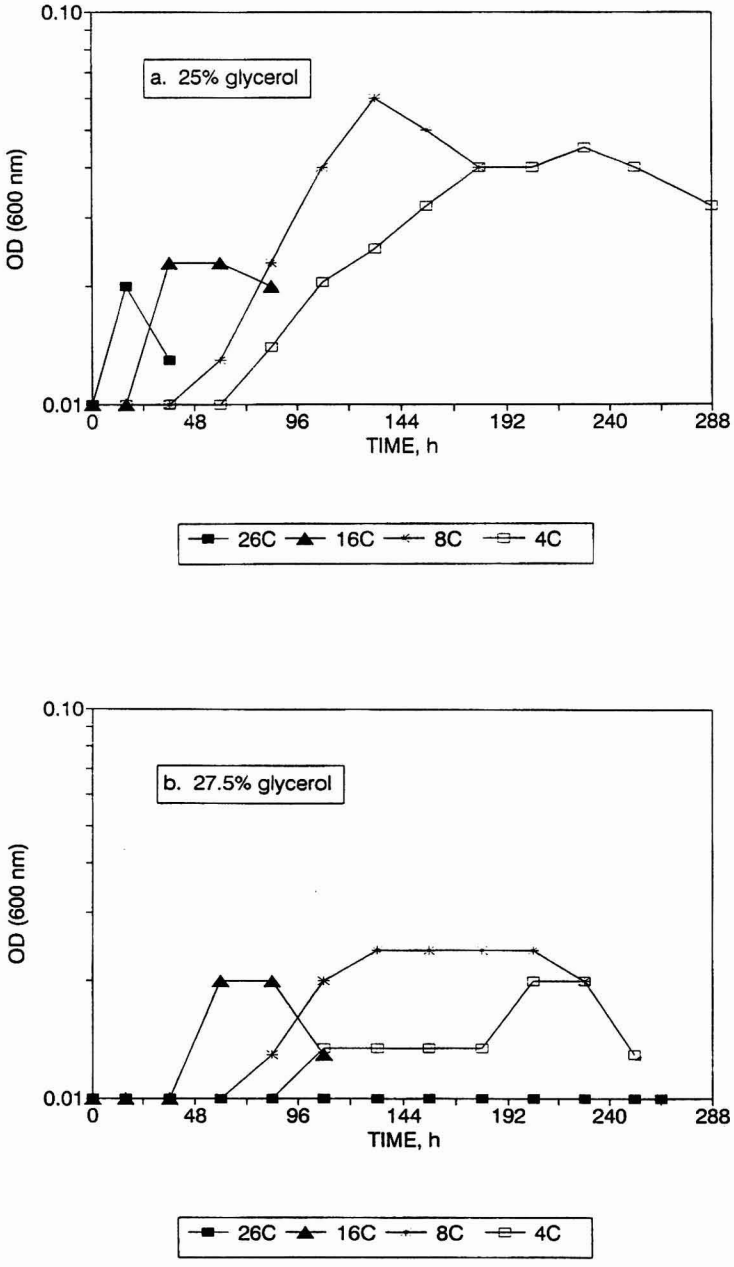
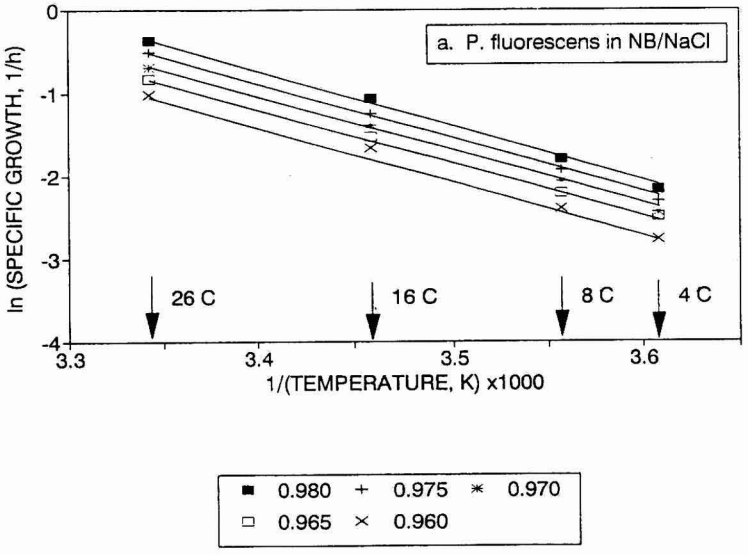


FIG. 2. EFFECT OF TEMPERATURE ON THE GROWTH OF *P. FLUORESCENS*
a. 25% glycerol (a_w = 0.947); b. 27.5% glycerol (a_w = 0.943).



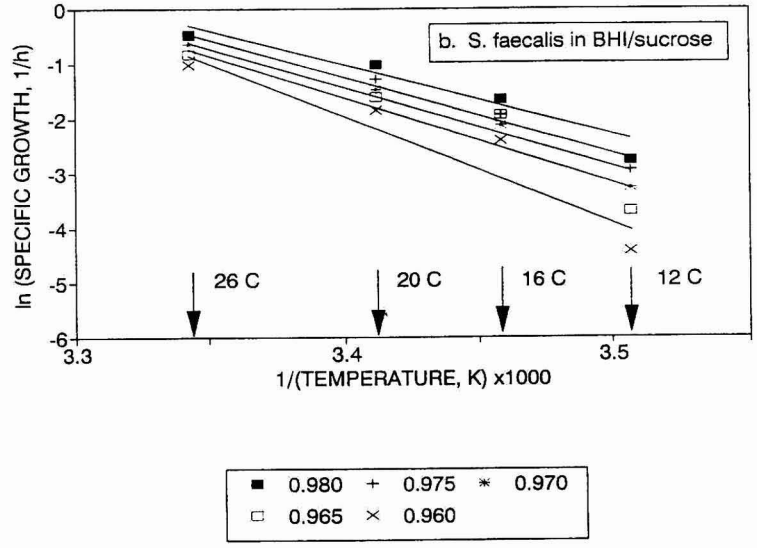


FIG. 3. EXAMPLES OF ARRHENIUS PLOTS FOR THE EFFECT OF TEMPERATURE O SPECIFIC GROWTH RATE

a. *P. fluorescens* in nutrient broth (NB) with a_w reduced with NaCl; b. *S faecalis* in brain hea infusion (BHI) with a_w reduced with sucrose.

TABLE 1. EFFECT OF a_w AND SOLUTE ON THE TEMPERATURE CHARACTERISTIC (kcal/g-mol) OF B. THERMOSPHACTA, *P. FLUORESCENS, S. TYPHIMURIUM, S. FAECALIS,* AND *S. AUREUS*[1]

	Water activity						
Strain	0.980	0.975	0.970	0.965	0.960	0.955	0.950
a. glycerol							
B. thermosphacta	---[2]	---	13.3 (.959)	13.5 (.963)	13.7 (.968)	14.0 (.974)	14.4 (.983)
P. fluorescens	13.0 (.950)	13.2 (.948)	13.4 (.945)	13.6 (.932)	13.7 (.934)	---	---
S. typhimurium	29.2 (.980)	30.7 (.961)	33.2 (.958)	37.8 (.935)	---	---	---
S. faecalis	---	---	31.5 (.969)	31.8 (.970)	32.2 (.971)	32.9 (.973)	33.9 (.975)
S. aureus	---	31.9 (.987)	32.9 (.990)	34.2 (.987)	36.3 (.982)	39.6 (.971)	46.7 (.940)
b. NaCl							
B. thermosphacta	---	---	11.7 (.983)	12.1 (.985)	12.6 (.988)	13.3 (.991)	14.8 (.944)
P. fluorescens	15.5 (.997)	15.6 (.997)	15.8 (.997)	16.0 (.997)	16.7 (.998)	---	---
S. typhimurium	27.3 (.988)	29.8 (.978)	34.2 (.985)	46.2 (.973)	---	---	---
S. faecalis	29.6 (.985)	31.6 (.985)	34.8 (.986)	41.5 (.982)	---	---	---
S. aureus	---	---	24.9 (.984)	25.7 (.955)	26.6 (.950)	27.8 (.944)	29.2 (.935)
c. sucrose							
B. thermosphacta	8.8 (.953)	9.1 (.961)	9.5 (.970)	10.1 (.979)	11.5 (.983)	15.7 (.976)	---
P. fluorescens	13.7 (.997)	14.2 (.997)	14.8 (.997)	15.8 (.995)	17.3 (.992)	---	---
S. typhimurium	21.3 (.986)	24.0 (.987)	34.7 (.985)	50.3 (.989)	---	---	---
S. faecalis	26.9 (.958)	28.1 (.953)	29.9 (.943)	32.7 (.927)	38.8 (.891)	---	---
S. aureus	---	23.8 (.935)	25.2 (.941)	27.2 (.948)	30.1 (.955)	35.2 (.959)	46.7 (.949)

[1] A complete set of Arrhenius plots can be found in Li, 1988. Values in parenthesis correspond to R^2 values used to estimate the degree of fit of the Arrhenius model.
[2] --- conditions not tested (high a_w) or too few data points (low a_w) for an Arrhenius plot.

concentration yielded different a_w's depending upon storage temperature (Li 1988; Li and Torres 1993a). In general, data for psychrotrophs fit the Arrhenius model better than for mesophiles, which in some cases show substantial deviations (Table 1).

The linearity of the Arrhenius plot is a matter of dispute as several authors have obtained conflicting results (Ingraham 1958; Ratkowsky *et al.* 1982). Ingraham (1958) and Janota-Bassalik (1963) have obtained straight lines, but some authors have observed curved plots (Ward and Cockson 1972) while others have obtained plots with two linear portions intersecting at a "critical temperature" (Mohr and Krawier 1980). The Arrhenius model was developed for elementary reactions and, while it is not surprising if sometimes it does not describe the effect of temperature on a complex biological process involving a variety of substrates and enzymes (Mohr and Krawier 1980), it is still widely used because of its sound theoretical basis (Almonacid-Merino *et al.* 1993a,b).

The slope of the Arrhenius plot is used to calculate an energy of activation. In the case of microbial growth some authors prefer the term "temperature characteristic" (Mohr and Krawier 1980; Reichardt and Morita, 1982). Reichardt and Morita (1982) have observed that this parameter is affected more by growth conditions and substrate availability than by the temperature range of interest.

The temperature characteristics for psychrotrophs were significantly lower than those for mesophiles (Table 1), an observation consistent with published values (Ingraham 1958; Mohr and Krawier 1980). As a_w decreases, the temperature characteristic increases, which means that more energy is required to overcome the growth barrier caused by low temperature and reduced a_w. Finally, in media with a_w reduced with glycerol, the effect of a_w reduction on the temperature characteristic was less pronounced as compared to NaCl or sucrose. For example, the temperature characteristic of *B. thermosphacta* in media containing NaCl was 11.7 kcal/g-mol at a_w = 0.970 and 13.3 kcal/g-mol at a_w = 0.955, i.e., a difference of 1.6 kcal/g-mol. However, using glycerol to control the a_w at the same two levels, the difference was only 0.7 kcal/g-mol but increased to 6.2 kcal/g-mol when using sucrose. This is another form of solute effect on microbial growth-a_w relationships and was observed to different extent with all test microorganisms.

CONCLUSIONS

The determination of the minimum a_w for the growth of five tested microorganisms in the 4–26C temperature range suggests *B. thermosphacta* as a good indicator of the microbial spoilage potential of RIMFs. The temperature characteristic (energy of activation) was found a useful indicator of temperature and solute-specific effects on microbial growth. The solute effect seems to be

related to physiological differences in the microbial response to a_w reduction and the solute used to reduce a_w. The temperature characteristic and minimum a_w for the growth of mesophiles was more temperature sensitive than those for psychrotrophs.

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BOOK REVIEW

***CLOSTRIDIUM BOTULINUM* ECOLOGY AND CONTROL IN FOODS.** Andreas H.W. Hauschild and Karen L. Dodds, Marcel Dekker, Inc., 270 Madison Avenue, New York, NY 10016, 1993. 440 pp. $135.00.

This book is a very readable review and an excellent reference for food microbiologists in industry, govemment and academia as well as others who are concerned with the microbial safety of foods. The editors build upon existing botulinal reviews by focusing the content on two areas, the ecology of the microorganism and control of growth and toxin formation in foods. The editors and chapter authors have created a comprehensive and cohesive text that updates the basic facts but also contains new and thought provoking material for the more cognizant reader. Each chapter is well-referenced for obtaining additional details.

The section on ecology includes a discussion of the current classification of *C. botulinum* and related toxin-producing species. There are extensive surveys of the worldwide occurrence of spores in nature and in foods. Of interest is a section on the incidence in honey and infant foods. The chapter on epidemiology catalogs the outbreaks of botulism, providing information on the country, number of cases, serotype, and type of food. The incidence and ecology of infant botulism receive extensive discussion and give food microbiologists an awareness of this still incompletely recognized problem.

In the portion of the book on botulinal control, an initial chapter examines the various agents of control: chlorine compounds, hydrogen peroxide, thermal inactivation, ionizing radiation, and ethylene oxide. Factors that prevent growth and toxin formation such as germinants, redox potential, pH, temperature, water activity and preservative are covered. Following chapters evaluate the occurrence clostridial spores and means to prevent growth and toxin formation in meat and meat products, fishery products, fruits and vegetables, dairy products and REPFEDS (refrigerated, processed foods of extended durability). The last is an excellent summary of the state of the art for this increasingly produced but microbially-suspect line of foods. Throughout these chapters are informative sections for even knowledgeable readers: the reliability of nitrite for control in meat products, effectiveness of CO_2 atmospheres, influence of other microorganisms, and role of lysozyme in thermal survival of spores. The

Journal of Food Processing and Preservation *17*, 319-320.

necessity to consider both toxin production and spoilage rates when deciding the safe storage protocol is explained.

Topics are not usually discussed by other reviews are the outbreaks in Northern countries from fermented (putrefied) native foods and stability of the toxin during storage or reheating. The final chapter summarizes efforts to model inactivation and growth/toxin formation. The latter includes models for probability of growth and for lag periods and growth rates. It concludes with views on the integration of models into HACCP programs. This reflects the ultimate concern of this entire book—to ensure that outbreaks of botulism from the consumption of commercially processed foods remain a rare or nonexistent event.

RICHARD C. WHITING, Ph.D.

GUIDE FOR AUTHORS

Typewritten manuscripts in triplicate should be submitted to the editorial office. The typing should be double-spaced throughout with one-inch margins on all sides.

Page one should contain: the title which should be concise and informative; the complete name(s) of the author(s); affiliation of the author(s); a running title of 40 characters or less; and the name and mail address to whom the correspondence should be sent.

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

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

Introduction: This should be brief and state the reason for the work in relation to the field. It should indicate what new contribution is made by the work described.

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

Results: The results should be presented as concisely as possible. Do not use tables *and* figures for presentation of the same data.

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

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

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

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

Journal abbreviations should follow those used in *Chemical Abstracts*. Responsibility for the accuracy of citations rests entirely with the author(s). References to papers in press should indicate the name of the journal and should only be used for papers that have been accepted for publication. Submitted papers should be referred to by such terms as "unpublished observations" or "private communication." However, these last should be used only when absolutely necessary.

Tables should be numbered consecutively with Arabic numerals. Type tables neatly and correctly as they are considered art and are not typeset. The title of the table should appear as below:

TABLE 1.
ACTIVITY OF POTATO ACYL-HYDROLASES ON NEUTRAL LIPIDS, GALACTOLIPIDS, AND PHOSPHOLIPIDS

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

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

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

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CONTENTS