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GAS PERMEATION AND THICKNESS OF THE SUCROSE POLYESTERS, SEMPERFRESH™ COATINGS ON APPLES[1]

H.J. PARK[2,3,5], J.M. BUNN[3], P.J. VERGANO[4] and R.F. TESTIN[3,4]

[3]*Department of Agricultural and Biological Engineering*

and

[4]*Department of Food Science*
Clemson University, Clemson, SC, 29634

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ABSTRACT

Oxygen permeability (OP) and water vapor permeability (WVP) of sucrose polyesters (SPE) coatings were measured and compared with other edible and plastic films. The OP of SPE coatings was higher than those of corn-zein films and plastic films such as polyethylene and polypropylene films but was similar to OP of cellulose film. The WVP of SPE coatings was lower than that of polyethylene film and more than 100 times lower than the values for cellulose and protein films. South Carolina grown apples (Red Delicious, Rome Beauty and Arkansas Black) were coated with solutions of SPE [0.6, 0.8, 1.0 and 1.2% (w/v)], and the thickness of the coatings measured. Coating thickness of SPE on the apples increased as concentration was increased. Coating thickness on red delicious was thicker than on the other varieties of apples for all concentrations of SPE.

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[2]Author Park was a research associate/assistant professor of the Department of Agricultural and Biological Engineering, Clemson University and is an assistant professor of the Department of Food Engineering, Mokpo National University, Mokpo, Korea.

[5]Send Correspondence to: Dr. Hyun J. Park, Adjunct Assistant Professor, Dept. of Agri. & Bio. Engineering, Clemson University, Clemson, SC 29634; TEL: 803-656-4052, FAX: 803-565-0338.

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INTRODUCTION

Sucrose polyesters (SPE) has been applied as coatings on fresh fruits such as apples, pears and bananas to extend their postharvest shelf-life since the 1980s. Lowings and Cutts (1982) reported SPE is an edible coating material that is nonphytotoxic, tasteless, odorless, and it is effective for preserving fruits. As commercially used, this coating material is a mixture of sucrose polyesters available under the trade names of "Semperfresh" and "TAL Pro-long" and is approved for coating fruits.

As a coating material it is a gas barrier that can modify internal gas composition and decrease the respiration rate in fruits. Banks (1984) applied SPE to bananas and found it modified the internal gas composition and retarded color change during storage. Smith and Stow (1984) reported that SPE coatings reduced yellowing and loss of firmness and markedly increased internal carbon dioxide level in apples (CV, Cox's Orange Pippin). Chu (1986) applied SPE to apples and reported that the coatings reduced softening of low O_2 stored "McIntosh" apple and controlled atmosphere stored "Delicious" apple. Santerre *et al.* (1989) reported that SPE treatment reduced "Golden Delicious" and McIntosh" apples ripening rate as observed by parameters such as color and texture. Nisperos-Carriedo *et al.* (1990) studied the retained flavor components in pineapples coated with SPE. Chai *et al.* (1991) applied SPE to Michigan apples ("Golden Delicious", "Ida Red" and "McIntosh") and found it delayed ripening and improved shelf-life during storage.

In these previous studies, little evidence was found to relate desirable gas permeation properties of SPE to coating thickness. Since it seems evident that gas permeation depends on the thickness of a coating on the surface of a fruit, this needs to be better understood to more effectively use SPE. Full success of SPE coatings for fruits will depend on applying an appropriate thickness, which will give a desirable internal gas composition over an extended time. If a coating is too thick, detrimental effects can result in fruits, including increased incidence of decay, anaerobic fermentation and off-flavor accumulation in tomatoes as reported by Park *et al.* (1994) and in apples as reported by Smith *et al.* (1987). Likewise, the effectiveness of a coating can be reduced if it is too thin. It was proposed that by knowing (a) gas permeation properties of edible coatings and fresh fruit skins, (b) respiration rates of the fresh fruits and (c) internal gas compositions of the fresh fruits, methods can be developed to better control the internal gas composition of bulky plant organs (Park *et al.* 1992). This entails applying an appropriate thickness of a coating material so that the postharvest shelf-life of fresh fruits can be extended during storage.

The objectives of this study were: (a) to measure gas permeability of SPE and (b) examine methods of applying and measuring coatings on South Carolina apples.

MATERIALS AND METHODS

Fruit

Red Delicious, Rome Beauty and Arkansas Black were purchased in winter from a local farmer in the Longcreek, South Carolina. They had been in cold storage for 2–3 months. Apples were sorted for uniform size and physical appearance and stored at 5C prior to application of sucrose polyesters (SPE) treatments. A total of 120 apples for each variety were divided into four groups and dipped in four different SPE solutions.

Treatment

Semperfresh™ powder (Inotek International Co., Painesville, OH) was used to prepare four different SPE solutions [0.6, 0.8, 1.0 and 1.2% (w/v)] as described by Santerre *et al.* (1989). Each solution (7 l) was placed in an open top container that was partially submerged in a water bath maintained at 25C. Groups of 30 apples were put in a wire mesh container (30 cm × 30 cm × 15 cm) and dipped by total submergence into the SPE solution for 15 s. The temperature of the water bath was recorded before and after dipping each group of apples. All treated apples were then dried at room temperature (23C) by blowing air over them with a table fan. Drying was completed in about 2 h. No thinning was observed in drying the apples.

Coating Thickness

Thickness of SPE coatings on apples was measured by taking a thin slice from coated fruits and observing its side view under a microscope (Leica Microstar IV Microscope, Model 410), which has a micrometer (each unit = 1.0×10^{-5}m) in eyepiece as described by Park *et al.* (1994). Two thin slices were taken from each apple. Thicknesses of the coating at five different points of each thin slice were averaged. A mean value of thicknesses of the two slices from each apple was calculated. Duncan's multiple range test was used to compare means for film thickness of coatings on apples with a level of significance of $\alpha = 0.05$ (SAS 1985).

Film Gas Transmission Rates

SPE films were prepared on polyethylene (PE) and polypropylene (PP) backings, which provide mechanical protection during measurement of gas permeabilities. SPE film solutions were poured onto polyethylene and polypropylene films attached to glass backing plates and dried at room conditions. The dried films were detached from the plate and test samples cut through the layers. An OX-TRAN 1000™ system (Modern Controls, Inc., Minneapolis, MN) was used to measure oxygen permeability through the combined films and the uncoated polyethylene and polypropylene films. These tests were run according to ASTM Standard Method D 3985-81 (ASTM 1989). A PERMATRAN-W600™ system (Modern Controls, Inc., Minneapolis, MN) was employed to measure water vapor permeability (WVP) through the combined films and the uncoated polyethylene and polypropylene films. These tests were according to ASTM Standard Method E 96-80 (ASTM 1987) on these same films. Unsupported SPE films were too weak to allow oxygen and water vapor permeabilities by these methods. For comparison, WVP of unsupported SPE film, detached from the combined films, was also measured by a cup method (Park and Chinnan 1993). Oxygen or water vapor permeabilities of SPE film were calculated from the following equation (Crank 1975):

$$P_{SPE} = X_2/((X_t/P_t) - (X_1/P_1)) \quad (1)$$

Where: P_{SPE}, P_1 and P_t are the permeability ((l or g) · m/m^2 · s · Pa) of SPE, plastic film (PE or PP) and double layers film (SPE + PE or SPE + PP), respectively; X_2, X_1 and X_t are the thickness (m) of SPE, plastic film (PE or PP) and double layers film (SPE + PE or SPE + PP), respectively.

Film Thickness Measurement

A hand-held micrometer (B.C. Ames Co., Waltham, MA) was used to measure film thickness to an accuracy of 0.000653 cm (0.25 mil). Five measurements were made on each test sample and a mean thickness reported.

RESULTS AND DISCUSSION

Gas Permeation of Sucrose Polyester (SPE)

Oxygen and water vapor permeabilities of SPE coatings as determined in this study are presented in Table 1 as are values for other edible coatings

TABLE 1.
OXYGEN AND WATER VAPOR PERMEABILITIES OF SEMPERFRESH™, SUCROSE POLYESTERS COATINGS

Film	Thickness (mm)	Permeability[1]	
		Oxygen	Water vapor
SPE[2]	0.065	5.50 ± 0.0003	1.00 ± 0.10
SPE[3]	0.042	2.10 ± 0.0001	0.42 ± 0.04
SPE[4]	0.029	-	0.44 ± 0.05
PE	0.038	1.80 ± 0.039	1.60 ± 0.04
PP	0.025	0.55 ± 0.005	0.65 ± 0.06
Edible coatings from the literature			
CZ[5]	0.1-0.3	0.36	116
MC[5]	0.04-0.07	2.17	92
HPC[5]	0.05	3.57	110
Cozeen[6]	0.09	0.89	407

[1]Values refer to mean and standard deviation; Unit of oxygen permeability is in $fl \cdot m/m^2 \cdot s \cdot Pa$ where f is an abbreviation of femto (10^{-15}); Unit of water vapor permeability is in $pg \cdot m/m^2 \cdot s \cdot Pa$ where p is an abbreviation of pico (10^{-12}); PE (polyethylene) n=3; PP (polypropylene) n=3.

[2]SPE is the value for Semperfresh™ film alone, calculated from a measurement made on Semperfresh™ film laminated on polyethylene (n=5) using by Equation (1).

[3]SPE is the value for Semperfresh™ film alone, calculated from a measurement made on Semperfresh™ film laminated on polypropylene (n=5) using by Equation (1).

[4]SPE film was detached from polyethylene film, and water vapor permeability measured by a cup method (Park and Chinnan, 1993).

[5]Park and Chinnan (1993); CZ (corn-zein); MC (Methyl cellulose); HPC (hydroxypropyl cellulose).

[6]Aydt et al. (1991).

reported in the literature. Oxygen permeability (OP) of SPE coatings was 1–3 times higher than those of polyethylene film and was 4–10 times higher than those of polypropylene film. OP of SPE coatings were similar to cellulose film values but were higher than those of protein edible coatings such as corn-zein

and Cozeen™. SPE coatings are very high water vapor barriers compared with other edible coatings. WVP of SPE coatings was lower than that of polyethylene film and more than 100 times lower than the values for cellulose and protein films. These high oxygen and water vapor barrier properties will make SPE coatings desirable for fresh produce as a replacement for wax (Risse *et al.* 1987; Segall *et al.* 1974).

Equation (1) was used to calculate permeabilities of oxygen and water vapor of the coatings. It is believed that the differences in OP and WVP of SPE laminated on polyethylene and polypropylene films can be attributed to differences in the thickness of SPE coatings. OP and WVP of hydrophilic films such as cellulose and protein films are a function of film thickness as reported by McHugh *et al.* (1993), Park and Chinnan (1993) and Pascat (1986). Several explanations have been supported for this effect. These include film swelling and different structure forming during WVP measurement (McHugh *et al.* 1993).

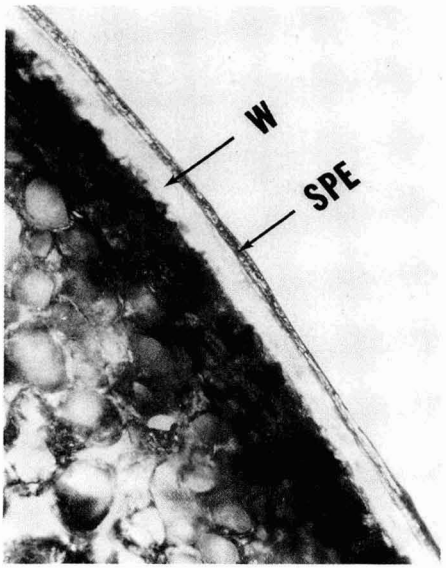

FIG. 1. A CROSS-SECTIONAL MICROGRAM (10 × 10) OF APPLE (RED DELICIOUS) COATED WITH SUCROSE POLYESTERS
W is the natural wax layer.
SPE is the sucrose polyester coating.

Coating Thickness on Apples

Measured SPE coating thicknesses on apples are shown in Fig. 1. Coating thickness was mainly affected by the concentration of the coating solutions. Coating thickness increased for all apple varieties as the concentration of SPE in the forming solution increased (Table 2). For example, a coating thickness of 0.58×10^{-5}m was measured for a 0.6% (w/v) solution on Red Delicious apples, and this increased to 1.32×10^{-5}m with a 1.2% (w/v) SPE solution. This is similar to the trend reported by Park *et al.* (1994) for thicknesses of corn-zein coating on tomatoes, which increased as the concentration of the solutions increased. Commercial recommendations for concentrations (w/v) of Semperfresh™ on apples range from 0.6% to 1.2% depending on the variety of apple by the Inotek International Company (Painesville, OH), a manufacturer of the Semperfresh™. Ripening of apples coated with higher concentrations of SPE was delayed more than apples treated with lower concentrations as measured by the persistence of green tissue color and increased tissue firmness and titrable acidity (Chai *et al.* 1991). The higher the concentration of a SPE solution, the thicker the coating produced on apples, and thicker coatings are higher gas barriers. Thicker coatings will also reduce the respiration rates of apples and delay the ripening process more than thinner coated apples. However, it may be noted that a coating that is too thick can cause physiological disorder by reducing internal gas composition in fruits (Smith and Stow 1987; Park *et al.* 1993).

TABLE 2.
SEMPERFRESH™, SUCROSE POLYESTERS, COATINGS ON APPLES

Apples	Coating thickness[1]			
	Semperfresh™ concentrations (%) (w/v)			
	0.6	0.8	1.0	1.2
Red Delicious	0.58 ± 0.34^{a}	0.70 ± 0.29^{b}	1.00 ± 0.25^{c}	1.32 ± 0.43^{c}
Rome Beauty	0.47 ± 0.20^{a}	0.61 ± 0.27^{b}	0.90 ± 0.23^{c}	1.02 ± 0.26^{d}
Arkansas Black	0.45 ± 0.14^{a}	0.58 ± 0.20^{ab}	0.64 ± 0.22^{b}	0.73 ± 0.29^{c}

[1]Unit of thickness is meter ($\times 10^{-5}$); values refer to mean and standard deviation (n = 30); thickness values within same variety of apple with different letter superscripts are significantly different as determined by Duncan's multiple range test $\alpha = 0.05$.

Coatings on Red Delicious apples were thicker than those on Rome Beauty and Arkansas Black for all concentrations of SPE solutions (Table 2). These thickness differences were concluded to have been caused by differences in surface characteristics of the apples. The observations in this study correlate with the findings of Chai *et al.* (1991) and Santerre *et al.* (1989) who reported that the effects of SPE coatings on apples differed with different varieties of apples. Chai *et al.* (1991) reported that SPE treatments improved consumer acceptability ratings for Golden Delicious and McIntosh apples, but had no significant improvement for Ida Red apples. These differences may have been caused by different coating thickness even through the apples were treated with the same concentration of SPE. Park *et al.* (1994) reported that the degree of color change and weight loss of corn-zein coated tomatoes depend on the coating thickness. Therefore, it may be concluded that the thickness of edible coatings on fruits should be controlled, and the effects of coatings should be understood to be related to coating thickness.

SUMMARY AND CONCLUSIONS

The oxygen permeability (OP) of sucrose polyesters (SPE) coatings was higher than those of corn-zein films and plastic films such as polyethylene and polypropylene films. The water vapor permeability (WVP) of SPE coatings was lower than those of corn-zein, methyl cellulose, hydroxypropyl cellulose and cozeen films by a significant amount, a factor of 0.01 or less. Coating thickness of SPE on the apples increased for all apple varieties as the concentration of SPE in the forming solution increased. Coating thickness on red delicious was thicker than on the other varieties of apples for all concentrations of SPE.

This study was also directed toward determining the optimal coating thickness of SPE on apples. The success of edible film coatings for fruits and vegetables will depend on the control of coating thickness because the transmission rates of oxygen and water vapor depend on the coating thickness. Quality and shelf-life characteristics such as color change, flavor loss, weight loss and decay incidence and sensory evaluations will be included in the next experiment on the effects of SPE on apples.

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DISPERSED PHASE CONCENTRATION EFFECT ON WATER VAPOR PERMEABILITY IN COMPOSITE METHYL CELLULOSE-STEARIC ACID EDIBLE FILMS

VIMAL SAPRU and THEODORE P. LABUZA[1]

Department of Food Science and Nutrition
University of Minnesota
1354 Eckles Avenue, St. Paul, MN 55108

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ABSTRACT

Edible films of methyl cellulose, stearic acid and polyethylene glycol, made by the emulsion method, were constructed with varying volume fractions of stearic acid. These films were evaluated for water vapor permeability. There was a significant decrease in the water vapor permeability through these films with increasing stearic acid volume fractions up to 22%. Subsequent increase in the stearic acid volume fraction resulted in an increase in the water vapor permeability. This was attributed to inadequate filling of the void volume within the stearic acid crystallites by methyl cellulose-polyethylene glycol matrix. Very high water vapor permeability values were obtained for pure stearic acid films. Scanning electron microscopy revealed intercrystallite air passages. An empirical mathematical model adequately expressed the change in permeability of these heterogeneous films up to 22% stearic acid fractions.

INTRODUCTION

Edible films are meant to reduce disposable packaging, wherever possible, and more importantly prolong storage life by resisting moisture migration and retarding gas transport. Kester and Fennema (1986) have presented a concise overview on the rationale for using edible films, formation procedures for these films, and types and characteristics of various films, along with a number of examples. Edible films may either be multilayered or may be composites made by drying of an emulsion (Kamper and Fennema 1984a,b, 1985; Kester and Fennema 1989a,b; Greener and Fennema 1989a,b; Hagenmaier and Shaw 1990,

[1]Author to whom inquiries shuold be addressed; Phone: (612)624-9701.

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1991; Koelsch and Labuza 1992). Avena-Bustillos and Krochta (1993) examined the effect of pH, calcium cross-linking and lipid content on the water vapor permeability of caseinate-based edible films. The present study is concentrated on methyl cellulose-stearic acid films made by the emulsion method. Methyl cellulose is the least hydrophilic of the water soluble cellulose ethers. Films made by the emulsion technique offer better control over film properties, higher mechanical strength and are easy to construct (Kamper and Fennema 1984a,b; Kester and Fennema 1989a,b). Traditionally, research in this area has been restricted to construction of edible film composites with different polymer substrates. Evaluation has been restricted to permeance/permeability comparisons under different relative humidity gradients and temperatures. The objective of this research was to evaluate the water vapor permeability as a function of the volume fractions of stearic acid and to apply a mathematical expression that would describe the behavior of these multicomponent edible films. Since a number of studies have published the water vapor permeability values at different lipid fractions for (Hydroxypropyl)Methylcellulose-stearic acid emulsion films, these films were chosen for this study.

MATERIALS AND METHODS

Film Composition

Methyl cellulose-stearic acid films were constructed by combining methyl cellulose (Sigma Chemical Co., St. Louis, MO), stearic acid (Henkel Chemical Co., Cincinnati, OH), polyethylene glycol (Fisher Scientific Co., NJ) as plasticizer, ethanol and water (2:1). The mixtures with different volume fractions of stearic acid (solid basis) were heated while blending on a hot plate stirrer at about 70C, deaerated in a vacuum oven, cast and dried in 90 mm diameter Plexiglas Dishes (Fisher Scientific Co., NJ). Formulations ensured polyethylene glycol:methyl cellulose ratio of 1:10. Film thickness for pure methyl cellulose film and emulsion films at different stearic acid volume fractions was between 0.24–0.28 mm. Thickness for pure stearic acid film was 1.35 mm since thinner films resulted in breakage during their construction.

Permeability Measurements

The film moisture permeability was measured using the standard cup method (E-96)(ASTM 1987). The film was mounted on a Twing-Albert® (Philadelphia, PA) cup filled with calcium chloride as the desiccant. The edges of the film

were sealed by pouring a molten blend of paraffin and microcrystalline wax along the edge of the cup. The cup was placed in a controlled environment of 25C and 75% RH. Weight gain was determined periodically over 60 h. Resistances to mass transfer in the gas phase on both sides were assumed to be negligible. Barrier permeability P at constant temperature was calculated from the expression (Karel *et al.* 1959; Biquet and Labuza 1988)

$$\frac{F}{A} = -\frac{P\,\Delta p}{\Delta x} \tag{1}$$

where F/A is the water vapor transmission rate [g/(day · m^2)] across a constant partial pressure gradient $\Delta p = P_1 - P_2$ and thickness Δx. Since this was a general comparison of permeability measurements made by different researchers, corrections for stagnant air gap height (McHugh *et al.* 1993) were not made to maintain consistency in the calculation method.

Moisture Diffusivity Measurements

Crank (1975) gives solutions of Fick's equation for unsteady state diffusion for different geometries. The solution for an infinite slab, which can represent a film, is indicated below:

$$1 - \frac{X - X_0}{X_e - X_0} = \frac{X - X_e}{X_0 - X_e} = \sum_{n=0}^{n=\infty} \frac{8}{(2n+1)^2 \pi^2} \exp^{-\frac{D(2n+1)^2 \pi^2}{4l^2} t} \tag{2}$$

where $X - X_0$ denotes the total amount of diffusing substance that has entered the sheet at time t and $X - X_e$ the corresponding quantity after infinite time, assuming a uniform initial moisture content X_0 in the region $-1 < \times 1 <$ and a constant surface concentration X_e. Simplifying assumptions include constant diffusivity and an isotropic homogeneous body. Thus for an anisotropic medium, as would be the case for an emulsion film, the solution would yield an effective diffusivity, D, assuming swelling does not occur to change film geometry (Mohney *et al.* 1988) at 75% RH. Moisture absorption data was generated in triplicate by measuring the weight gain (± 0.001g) of the film maintained at 25C and 75% RH. The experimental data was fitted to Eq. 2 for determining the optimum diffusion coefficient by nonlinear regression using the Marquardt-Levenberg method (Press *et al.* 1986).

Scanning Electron Microscopy

Scanning electron microscopy was performed on Hitachi 450 scanning electron microscope. The sample was broken to expose a fresh cross section, mounted on aluminum stubs, and sputter coated with gold.

RESULTS AND DISCUSSIONS

Figures 1 and 2 show the representative moisture absorption curve for pure methyl cellulose and pure stearic acid films fitted by Eq. 2 for an infinite slab. The predicted diffusivity was 1.34×10^{-9} m^2/h for the pure methyl-cellulose film, while the diffusivity for pure stearic acid film was 3.4×10^{-8} m^2/h. This was surprising since one expects a much lower diffusion coefficient of moisture in pure lipid.

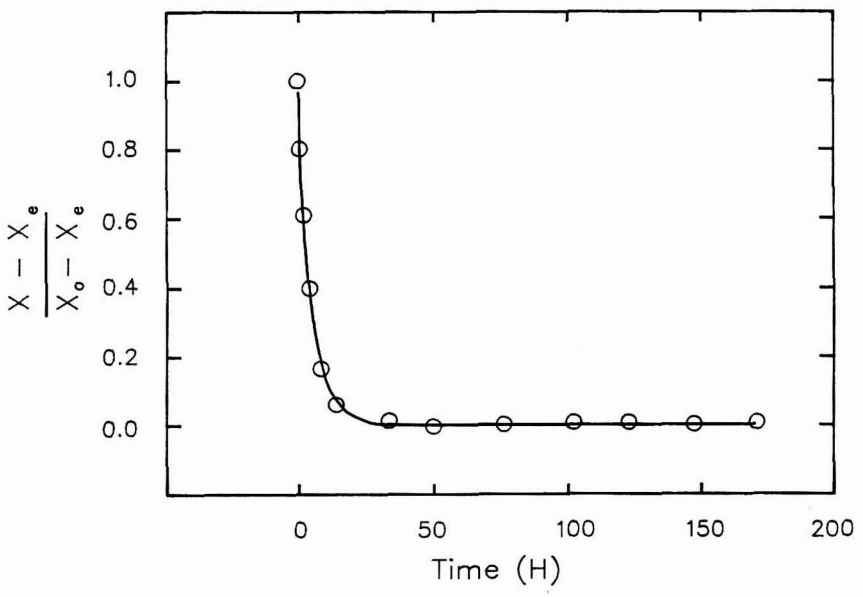

FIG. 1. MOISTURE ABSORPTION CURVE FOR METHYLCELLULOSE FILM
___ Best fit line, o experimental data.

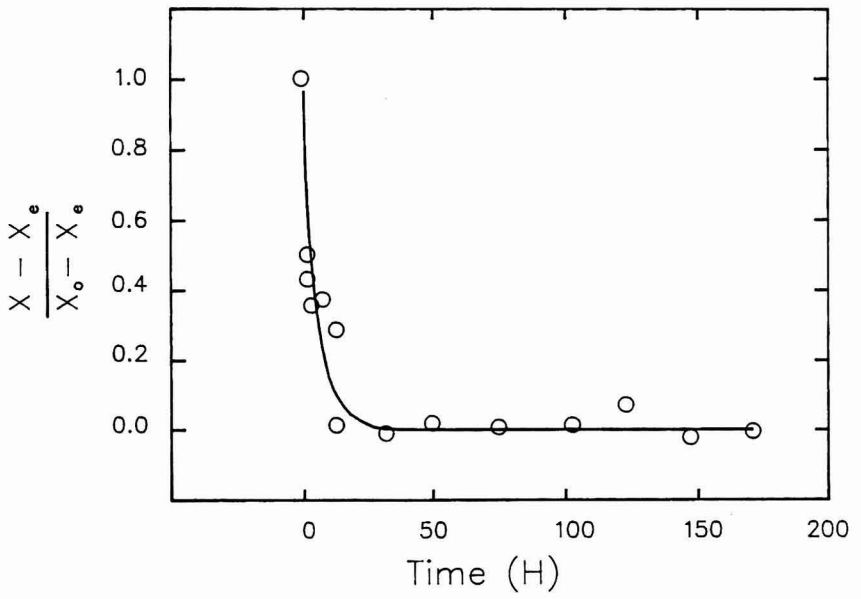

FIG. 2. MOISTURE ABSORPTION CURVE FOR STEARIC ACID FILM
___ Best fit line, o experimental data

The measured water vapor permeabilities of methyl cellulose and for emulsion films made with varying volume fractions of stearic acid are shown in Table 1 and compared to prior published work. These values are similar to those obtained by other researchers at lower volume fractions of stearic acid. Any differences in the permeability values can be attributed to the use of hydroxypropylmethylcellulose by Kamper and Fennema (1984b) and Hagenmaier and Shaw (1990) and also to the fact that measurements were made at different relative humidity conditions. However, the permeability of pure stearic acid film resulted in very high values consistent with the high water vapor diffusivity. Scanning electron microscopy of the pure stearic acid film revealed crystal aggregates with significant interspatial air pockets as shown in Fig. 3, explaining the anomaly in the results of both the permeability and diffusivity values. Data of other researchers (Table 1) indicated an increase in permeability above a certain volume fraction of dispersed lipid phase and was suggested to be due to the difficulty in film formation. Kamper and Fennema (1984b) found that permeability increased above 22% volume fraction of stearic acid, while Hagenmaier and Shaw (1990) found that the increase in permeability occurred above a volume fraction of 46%. Koelsch (1992) found that the increase in permeability occurred above a volume fraction of stearic acid of 40%.

TABLE 1.
MOISTURE PERMEABILITY OF (HYDROXYPROPYL) METHYL CELLULOSE-STEARIC ACID BICOMPONENT FILMS AS A FUNCTION OF LIPID VOLUME FRACTION

This study 25 C, 0-75%RH		Kamper and Fennema, 1984b 25 C, 0-85%RH		Koelsch, 1992 23 C, 12-35%RH		Hagenmaier and Shaw, 1990 27 C, 0-85%RH	
V_d[1]	P[2]	V_d	P[3]	V_d	P[2]	V_d	P[3]
0	50.21	0	35.18	0	47	0	48
0.03	39.78	0.027	10.17	0.097	27.95	0.024	31
0.06	23.36	0.059	4.32	0.176	18.91	0.163	2.1
0.10	17.30	0.140	0.55	0.244	4.232	0.274	0.5
0.14	9.04	0.185	0.40	0.303	0.969	0.443	0.16
0.19	31.24	0.257	0.21	0.348	0.51	0.494	0.14
0.26	30.47			0.391	0.63	0.603	0.23
1.00	183			0.428	0.78		
				0.461	0.61		

[1]Volume fraction of lipid (Dispersed phase)

[2]Permeability (g_{water}.mil)/(m^2.day.mmHg) for methylcellulose-stearic acid film

[3]Permeability values for hydroxypropylmethylcellulose-stearic acid films.

It can be presumed that the heating and drying steps during film construction play an important part in determining the permeability of these films, due to the crystal formation of the lipid. Hagenmaier and Shaw (1990), who prepared films at the highest volume fractions of stearic acid and had the lowest permeability values, have taken extensive care in preventing stearic acid crystallization by controlling the drying temperature. This probably also prevents formation of large stearic acid crystallites before the stearic acid is captured within the methyl cellulose continuous layer and thus prevents formation of a significant number of interspatial channels through which water vapor can pass.

Mathematical Model

It was attempted to fit all the permeability data with a simple empirical model to obtain the permeability of the film for different volume fractions of stearic acid. Only the decreasing values for permeability were used for this analysis. This behavior is described by the equation:

$$P_{eq} = P_d^{(V_d)} \; P_c^{(1-V_d)} \tag{3}$$

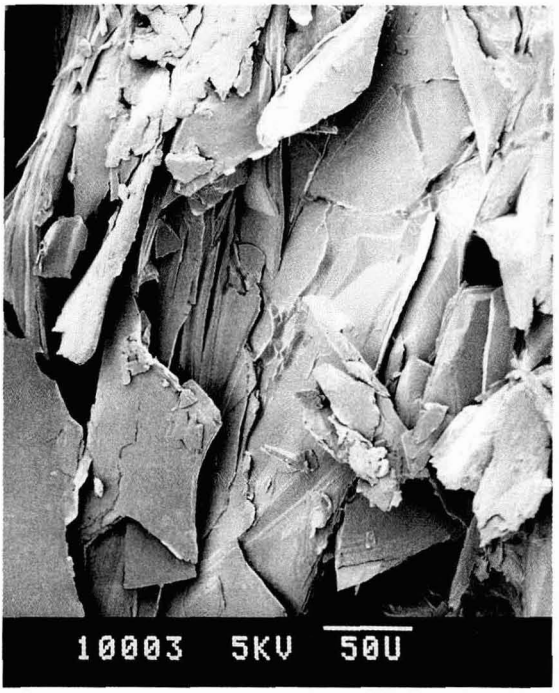

FIG. 3. SCANNING ELECTRON MICROSCOPE PICTURE OF STEARIC ACID FILM CROSS SECTION

where P_{eq}, P_d and P_c are the equivalent film permeability, dispersed phase permeability and the continuous phase permeability's respectively, and V_d is the volume fraction of the dispersed phase. This model is often used to evaluate physical properties of randomly distributed heterogeneous systems (Vagenas and Karathanos 1991). Figure 4 shows the results using data from all four studies. For permeability of the continuous phase P_c the average value of 45 g.mil/m^2 . day.mmHg was used. The best-fit value for P_d, the permeability of pure stearic acid crystallites was obtained by fitting Eq. 3 to the permeability data using the Marquardt-Levenberg nonlinear regression method (Press *et al.* 1986). The predicted value for P_d, the permeability of pure stearic acid crystallites obtained is low (8×10^{-5} g.mil/m^2.day.mmHg)) and is consistent with the permeability values predicted for a number of moisture resistant films. In the event of

keeping P_c an unknown in the regression procedure, the best-fit value for this parameter converges to 43(g.mil)/(m^2.day.mmHg), a value very close to the average value obtained from published data.

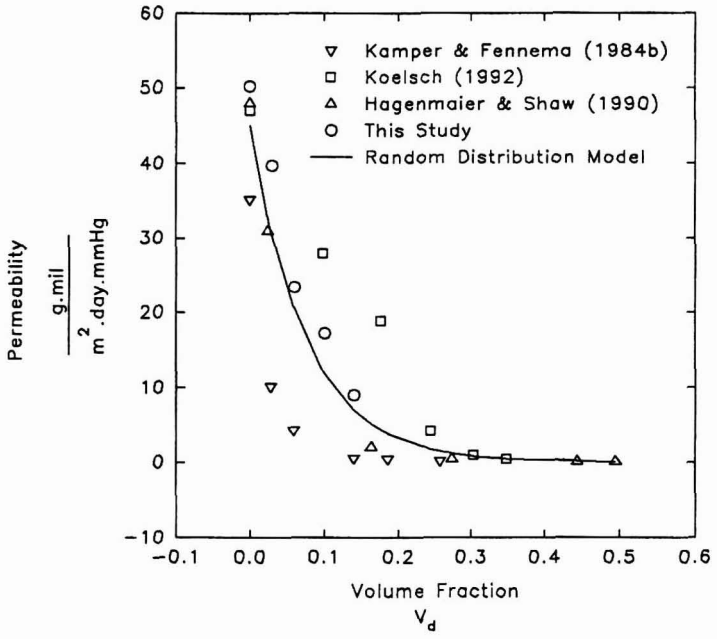

FIG. 4. EXPERIMENTAL DATA INDICATING DECREASING PERMEABILITIES
Selected from Table 1 and the best-fit line obtained by nonlinear regression of the data to Eq. 3

Thus it can be presumed that lipid crystallites of complex morphology are held within the methyl-cellulose matrix, and permit a significant drop in permeability at lower volume fractions. However as the volume fraction of the dispersed phase increases, there is inadequate filling of the void volume within the stearic acid crystallites by methyl cellulose-polyethylene glycol matrix, thereby providing open pores with high moisture diffusivity, thus accounting for the increase in the permeability. The extent of this interface is primarily dependent upon the volume fraction of the dispersed phase, plasticizer content, size of the crystallites, cooling and drying rates and film thickness. The random model describes the permeability behavior at different volume fractions of the dispersed phase and can be a useful tool in designing edible films with desired properties.

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QUALITY LOSS OF DOUBLE CONCENTRATED TOMATO PASTE: EVOLUTION OF THE MICROBIAL FLORA AND MAIN ANALYTICAL PARAMETERS DURING STORAGE AT DIFFERENT TEMPERATURES

P. VILLARI[1], P. COSTABILE[1], G. FASANARO[1], F. De SIO[1], B. LARATTA[1], G. PIRONE[2], and D. CASTALDO[1,3]

[1]*Department of Fruit Juices and Tomato Products*

and

Department of Microbiology
Stazione Sperimentale Industria Conserve Alimentari in Parma
Via Nazionale 121/123,84012 Angri (SA), Italy

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ABSTRACT

This study deals with nonsterile canning of double concentrate tomatoes (≈30° Brix) stored for 210 days at three different temperatures (4, 10 and 25C) in 200 kg drums. The evolving analytical composition (soluble solids, total solids, glucose, fructose, pH, total acidity, volatile acidity, citric acid, malic acid, succinic acid, hydroxymethylfurfural (HMF) and color parameters) that the product underwent during storage was dependent both on storage temperature and on different aerobic levels within the drum (top and bottom sections). The microbial profile (yeast, lactic acid bacilli and molds) was correlated with many important metabolites (D- and L-lactic acids, ethanol, acetic acid and diacetyl). The results indicate that the increase of these substances is dependent both on storage temperature as well as the oxygen tension within the drums.

Taken all together, the analytical findings offer a great help in evaluating the quality of semifinished tomatoes. We also found that lactic bacteria grow rapidly at 25C and after 15 days their number from both sampling areas in the drums (i.e., 10 cm below the sample surface and 15 cm above the bottom of each drum) is already greater than 10^5 cfu/ml. At 10C, 30 days were needed to reach such a cell concentration, and after 45 days the level reaches 10^7–10^8 cfu/ml. By contrast, at 4C there were differences between top and bottom sampling areas. In the top area, 10^5 cfu/ml was reached after 60 days, while for the bottom area this was reached after 120 days. Regarding yeast at 25C, the

[3]To whom correspondence should be sent.

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cfu/ml values were 10^5–10^6 in both areas of the drum after the 60th storage day. At 10C the behavior was the same: about 10^5 cfu/ml had been found after 30 days. Finally, at 4C the yeast reached 10^5 cfu/ml after 45 days in both sampling areas. Regarding mold, no growth in the sampling areas was seen.

INTRODUCTION

The temporary storage of semifinished tomatoes is usually brought about by sterile packaging of pulp, diced, double and triple tomato concentrates. Among the numerous advantages of this technology, we cannot fail to mention that during the canning season the preparation of semifinished and reworked product can be kept separate. The reworked product is essentially used to make sauce for canning. In the case of double and triple tomato concentrates, however, because of the high costs for sterile production it is still the practice in some regions of Italy and other Mediterranean countries to store partially refined tomatoes in 180–220 L plastic drums under nonaseptic conditions.

This semifinished product has found a good market above all because of the low cost, and it is often blended with other costlier semifinished tomatoes of good quality (Leoni and Bellucci 1980).

The technology behind packaging a semifinished product requires that the fruit be canned in nonsterile drums at relatively low temperature (65–70C). In addition, since the drums cannot be closed immediately because their walls undergo mechanical stress during cooling these products are subject to easy contamination. What is more, the superficial storage with salt (under widespread use) quickly alters the product. Moreover it is a more or less diffuse practice to store this product on truck loading grounds at the processing plant where, in absence of properly sealed cover, the product is exposed to the high temperatures usually occurring in these regions during summer (the season coinciding with tomato production and refinement).

Technical-scientific studies regarding these nonsterile products are scarce, especially those concerning variations of composition correlated with taste and smell changes. There is also scarce analytic data for better quality blend and for technological data about the best storage time and temperature to limit compositional changes.

Based on these considerations, this first study attempts to correlate the fermentation profile (microbial growth) with the composition (metabolite production) found in these semifinished products and to evaluate this change at three different storage temperatures. Finally, we evaluated the compositional difference between the same product at the bottom of a drum with the product at the top of a drum, where the oxygen tension is presumably higher.

MATERIALS AND METHODS

Tomato Samples

Six drums of tomato concentrate (≈30° Brix), produced and packaged by CPC. S.P.A., Castel S. Giorgio (SA), Italy, were used in the present study. The drums were first washed with detergents and then rinsed with tap water. They were finally treated with saturated steam for 1 min. The product was previously mixed in a tank to guarantee homogeneity and then filled at 68–70C.

Two drum lots each were stored at three different temperatures (4, 10 and 25C) for a total of 210 days. Two samples of tomato concentrate were drawn from each drum at various storage times. These were taken 10 cm from the top surface, and 15 cm from the bottom by a 4 cm cylindrical probe (Fig. 1). A 3 cylinder probe, 4 cm in diameter, was used to guarantee that the samples were evenly represented. The probe was sterilized by immersion in an alcohol solution of Iosan (Ciba-Geigy) and passed through a flame immediately before sampling.

Microbiological Analysis (Lactic Acid Bacteria, Molds and Yeasts)

Lactic acid bacteria cell counts were carried out on Rogosa Agar (Oxoid) by pour plate technique; petri plates were then incubated at 30C for 24–48 h (Rogosa *et al.* 1951). Then catalase tests and microscopic observations were carried out. The catalase-negative bacterial colonies were counted and considered as lactic acid bacteria. Yeast and mold counts were carried out in Malt Extract Agar (Oxoid) acidified to pH 4.0 with 50% citric acid solution sterilized by filtration; petri plates were then incubated at 30C for 48–72 h (Galloway and Burgess 1952); colonies were finally observed by microscope.

The cellular growth velocity is defined by $r_x = dX/dt$ where X is the cellular concentration of the microorganism (expressed as cfu/ml) and t is the time. From the ratio between r_x and X at various sampling time, one calculates the specific growth velocity, expressed as $\mu(\text{time}^{-1}) = (dX/dt) \cdot (1/X)$.

Analytical Determinations

For proximate analyses, the soluble solids, expressed as °Brix, were determined by measuring the refractive index at 20C. The total solids were determined by oven drying 10 g of sample, under vacuum at 70C, to constant weight. Titratable acidity (total acidity), expressed as citric acid monohydrate, was determined by titrating a 10 g sample to end-point pH 8.1 with 0.1 N

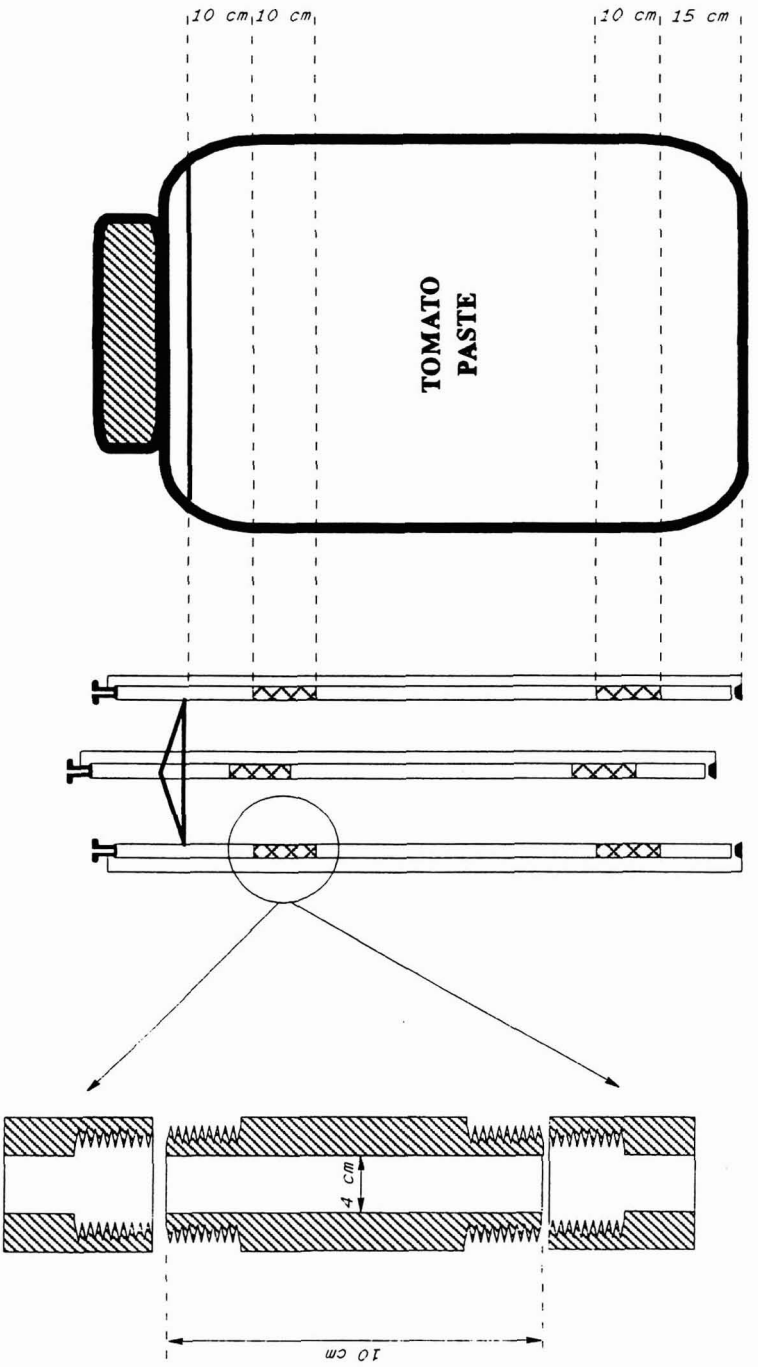

FIG. 1. SCHEMATIC DIAGRAM OF CYLINDRICAL PROBE

NaOH, according to Metodi Ufficiali di Analisi delle Conserve Vegetali (MUACV 1961). The volatile acidity was determined according to International Federation of Fruit Juice Producers method 5 (IFFJP 1962) calculated as acetic acid and expressed in g/100. The pH was determined potentiometrically at 20C by a Crison pH meter TT2050. Total sugars were determined by the MUACV (1961) procedure. The hydroxymethylfurfural (HMF) content was determined by reference to a standard curve according to IFFJP (1972) method 12. Briefly, the determination principle is that HMF reacts, like various other aldehydes, with barbituric acid and p-toluidine forming a red-colored compound. The intensity of the red color (read in a spectrophotometer at 550 nm) depends on the amount of HMF and can therefore be used as the basis of a quantitative colorimetric determination. D- and L-lactic acids, ethanol and acetic acid were determined by enzymatic methods (Boehringer Mannheim Biochemicals 1980). Luminance (L* value), redness (a* value) and yellowness (b* value) were measured using a tristimulus Hunter colorimeter model D25A. Standard plate N° C20-2105 with Hunter L* value of 25.8, a*value of 28.6 and b* value of 12.9 was used as a reference. The Hunter color values were measured at a soluble solids level of 8.2%.

Sample Preparation for Diacetyl Determination

The diacetyl was determined spectrophotometrically. Tomato paste (10 g) was diluted with 200 ml of H_2O. The prepared sample was distilled at 100C and the first 25 ml of distilate was collected. α-Naftol (5 ml) in isopropyl alcohol (obtained by dissolving 5 g of α-naftol in 100 ml of 95% isopropyl alcohol) and 2 ml of creatine solution (from 20 g of KOH and 0.15 g of creatine in 50 ml of H_2O) were added to 10 ml of distillate and shaken for 15 min. The red color produced was measured photometrically against air at 545 nm and compared to a standard curve obtained using diacetyl as a standard (standard curve ranged 0–3 mg/kg).

Sample Preparation for Sugar Determination (Glucose and Fructose): HPLC Analysis

Tomato paste (10 g) was diluted to 50 g with distilled H_2O and clarified by centrifugation at 12000 × g for 15 min. The clarified extract was filtered through 0.45-μm Millipore filters. A sample of 20 μl was used for analysis. A Merck Lichrosorb NH_2 (10 μm) cartridge was used. The eluent consisted of acetonitrile-water (80:20), which was degassed and filtered through a 0.22 μm Millipore filter. A Waters-Millipore 600E liquid chromatograph was employed

and a differential refractometer (Waters-Model 410) was utilized as a detector for sugar analysis.

Sample Preparation for Acid Determination (Citric, Malic and Succinic): HPLC Analysis

Tomato paste (30 g) was diluted to 50 g by H_2O and clarified by centrifugation at 12000 × g for 15 min. The clarified extract was filtered through a 0.45 μm Millipore filter; l0 ml were chromatographed through a cation-exchange column [AG-I-X8, (HCOO−) Bio-Rad] and washed with water to a total volume of 100 ml.

The organic acids were eluted with 6M formic acid (about 130 ml), collected, and evaporated. The dry samples were recovered with water (10 ml) and filtered through a 0.45 μm Millipore filter before HPLC analysis.

A Merck Lichrosorb RP-18 (10 μm) cartridge was used for HPLC analysis. The eluent consisted of water adjusted to pH 2.4 with H_3PO_4 at a flow rate of 2.0 ml/min. A Waters 410 differential refractometer was used as detector.

Statistical Analysis of Results

Analysis of variance (Snedecor and Cochran 1980) was used to determine differences in mean values obtained from results of six determinations in duplicate for each sample. Significance was determined at $P = 0.05$.

RESULTS AND DISCUSSION

Microbial Growth During Storage

The number of lactic bacteria and yeast at different storage temperatures is shown in Table 1. At the starting time, the numbers of yeasts and lactic bacteria both fell within the range of 0–10 cfu/ml.

Influence of Storage Conditions on Lactic Bacteria Levels. At 4C (Table 1), for storage time exceeding 120 days, the concentration of lactic acid bacteria in both sampling areas (top and bottom drum) was greater than 10^5 cfu/ml. At shorter storage time, the lactic acid bacterial growth-curve was different. In fact, for top level samples, 10^5 cfu/ml were already present at about 60 days and remained almost constant until the end of storage. In contrast, in the bottom

TABLE 1.
LACTIC ACID BACTERIA AND YEASTS COUNTS FROM TOMATO PASTE STORED AT 4, 10 AND 25C

Storage Time (Day)	Sampling Zone	Lactic Acid Bacteria[c]			Yeast[c]		
		4 C	10 C	25 C	4 C	10 C	25 C
0		< 10	< 10	< 10	< 10	< 10	< 10
15		2.4×10^1	2.6×10^3	1.9×10^6	1.8×10^1	1.3×10^3	6.4×10^3
30		4.0×10^1	1.3×10^3	2.0×10^4	5.8×10^2	4.0×10^4	6.4×10^3
45	T	1.4×10^4	4.0×10^7	6.0×10^5	3.0×10^5	5.0×10^4	2.6×10^4
60	O	1.0×10^5	2.5×10^8	1.2×10^5	2.2×10^6	1.2×10^7	3.5×10^5
75	P[a]	1.0×10^5	2.0×10^8	2.0×10^4	2.6×10^6	1.6×10^6	2.8×10^5
90		1.3×10^5	1.2×10^8	6.0×10^4	1.2×10^6	1.4×10^7	5.6×10^5
105		3.6×10^5	4.4×10^7	8.0×10^3	8.0×10^4	2.6×10^6	7.3×10^5
120		7.0×10^5	3.0×10^7	8.0×10^3	8.0×10^5	4.0×10^6	2.2×10^5
180		2.6×10^6	1.4×10^7	1.1×10^5	1.4×10^6	1.2×10^6	1.7×10^5
210		5.4×10^6	9.3×10^6	1.2×10^3	3.0×10^6	1.9×10^6	3.9×10^6
0		<10	<10	<10	<10	<10	<10
15		3.6×10^1	5.2×10^2	4.4×10^5	2.2×10^2	4.8×10^2	8.8×10^3
30	B	1.0×10^2	1.2×10^4	8.0×10^3	6.6×10^2	1.4×10^5	1.2×10^3
45	O	1.2×10^4	4.0×10^7	8.0×10^6	1.0×10^5	2.2×10^5	5.4×10^4
60	T	3.0×10^4	1.2×10^8	7.0×10^7	1.8×10^4	1.0×10^5	4.4×10^6
75	T	2.0×10^4	4.0×10^8	4.0×10^5	4.0×10^4	2.6×10^7	7.0×10^5
90	O	1.3×10^4	4.0×10^7	3.0×10^4	6.8×10^4	6.0×10^6	8.0×10^5
105	M[b]	1.0×10^4	3.2×10^7	1.4×10^4	2.2×10^4	8.0×10^6	2.6×10^5
120		2.8×10^5	9.0×10^7	3.4×10^4	9.0×10^4	3.0×10^6	5.4×10^4
180		1.3×10^6	1.0×10^8	1.0×10^4	2.6×10^6	1.0×10^6	1.1×10^5
210		4.8×10^6	2.1×10^7	3.9×10^4	1.2×10^6	1.8×10^6	1.1×10^6

[a] The sample was taken at 10 cm from the drum top. [b] The sample was taken at 15 cm from the drum bottom.
[c] The counts are expressed as cfu/mL

level samples, lactic acid bacteria concentrations remained constant between 45 days ($1.2\ 10^4$ cfu/ml) and 105 days ($1.0\ 10^4$ cfu/ml) and rose to 10^5 cfu/ml at 120 days and thereafter.

This trend was probably due to composition changes in the substrate occurring during storage. In these conditions the growth and inactivation of various strains are influenced by the type and concentration of metabolites produced. Since some strains are rapidly inactivated, others may also develop because of decreased microbial competition.

At 10C (Table 1) there appears to be no substantial difference between growth curves of lactic acid bacteria in the top storage samples compared with the bottom ones. 10^3–10^4 cfu/ml are present at about 30 days. As seen in Table 1, the bacteria growth curve continues to rise until 45 days and then levels off (10^7–10^8 cfu/ml) until the end of storage.

At 25C (Table 1), the growth of lactic acid bacteria was rapid. At 15 days the concentration reached 10^5 cfu/ml for both samples (top and bottom sites of the drum). The findings in Table 1 also show that at storage time exceeding 60 days the concentration of lactic acid bacteria in the top and bottom samples tended to decrease with storage temperature until leveling off within a range of 10^3–10^4 cfu/ml. These findings show that the lactic acid bacteria growth-curve, as predicted (Kandler and Weiss 1986), is not only dependent on storage time, but also and primarily on more aerobic conditions that probably occur in the top of the drum compared to those occurring at the bottom. These findings are in agreement with those reported by Lucey and Condon (1986) who found a higher rate of growth under aerobic conditions using some strains of *Leuconostoc* and with a greater production of acetate compared to anaerobic conditions.

Influence of Storage Conditions on Yeast Levels. At 4C (Table 1) the yeast growth-curves were identical (approximately 10^5 cfu/ml) after 45 storage days in the top and bottom areas of the drum. Instead, at higher storage temperature, the difference between top and bottom drum areas become relevant. In fact, at the top area of the drum, the number of yeasts continued to rise after 45 days until reaching approximately 10^6 cfu/ml, and then remained constant until the end of storage. By contrast, at bottom area of the drum, the yeast numbers remained almost constant, at about the value found on the 45th storage day, and only at the end of the storage reached 10^6 cfu/ml.

At 10C (Table 1), the difference in the number of yeasts is still higher than before. In fact, the content of yeasts after 30 storage days was about 10^4 cfu/ml in the upper area of the drum, and this is substantially lower than observed at the same storage time in the bottom sampling area (about 10^5 cfu/ml). The concentration of yeasts in both sampling areas became constant at 10^6 and 10^7 cfu/ml after 75 days of storage and remains so for the duration of the storage period. Finally, at 25C, the data reported in Table 1 show immediate growth in both the upper and lower areas of the drum with values reaching 10^3 cfu/ml at the 15th day and remaining between 10^5 and 10^6 cfu/ml after 60 days of storage.

Metabolite Production and Specific Growth Rate. To determine the influence of different metabolites produced during storage on the growth of yeasts and lactic acid bacteria, Fig. 2–7 report the specific growth rates (day^{-1}) as influenced by ethyl alcohol, acetic acid, diacetyl and lactic acids D-L.

The first finding to emerge from examination of the data reported in Fig. 2–4 is the varying level of ethanol produced during the 210 days of storage. There was a lower production at 4C (maximum concentration 9 g/kg) compared to the product stored at 10C (maximum concentration 32 g/kg) and at 25C (maximum concentration 38 g/kg).

It is noteworthy that the inhibition of yeast growth seen at different temperatures occurred almost always at ethanol levels reaching 4g/kg. The only exception is the product stored at 4C, which presents inhibition of the lower area sample (Fig. 2) with ethanol levels nearing 2g/kg. Generally speaking, the accumulation of ethanol from the fermentation of sugars by yeast does not appear to have the same inhibitory effect on lactic acid bacteria. As shown in Fig. 5-7, these latter had a lowered specific growth rate at different times, which seems to be coupled more with the ethanol than sugar level.

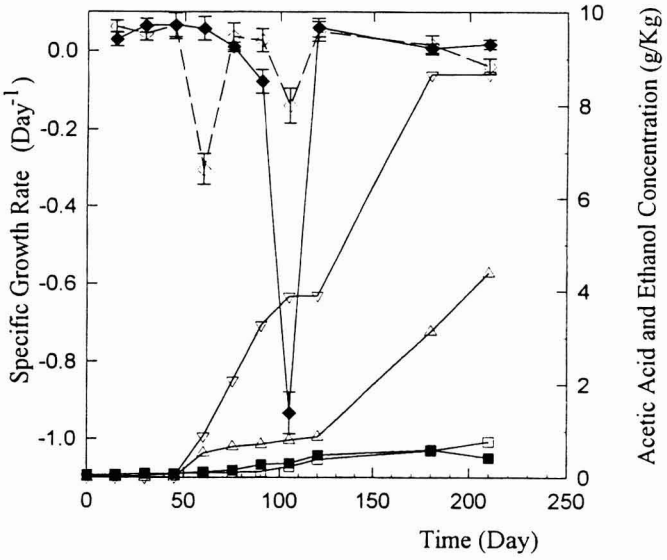

FIG. 2. EVOLUTION OF YEAST SPECIFIC GROWTH RATE, OF THE ACETIC ACID AND THE ETHANOL PRODUCT ON TOMATO PASTE STORED AT 4C
(◆) yeast specific growth rate (top), (◇) yeast specific growth rate (bottom), (∇) ethanol production (top), (Δ) ethanol production (bottom), (■) acetic acid production (top), (□) acetic acid production (bottom).

At 25C, inhibition of lactic acid bacteria in the top and bottom areas was almost immediate, the maximum occurring at about 25-30 days (Fig 7). At 10C the maximum inhibition in the top area of the drum occurred at approximately 90 days, while in the bottom area it occurs at about 75 days (Fig 6). At 4C (Fig. 5), the specific growth velocity in the top and bottom areas was almost always greater than zero; indeed, there appears to be no inhibition during storage.

Examination of the analytical data in Tables 2,3 and 4 clearly reveals that the fermentation of all samples stored at different temperatures features an increased Fru/Glu ratio and a lowered sugar quotient (SQ% = ((Fru+Glu)/total solids)) × 100). The latter parameter (SQ%) appears to be linked to a slowed

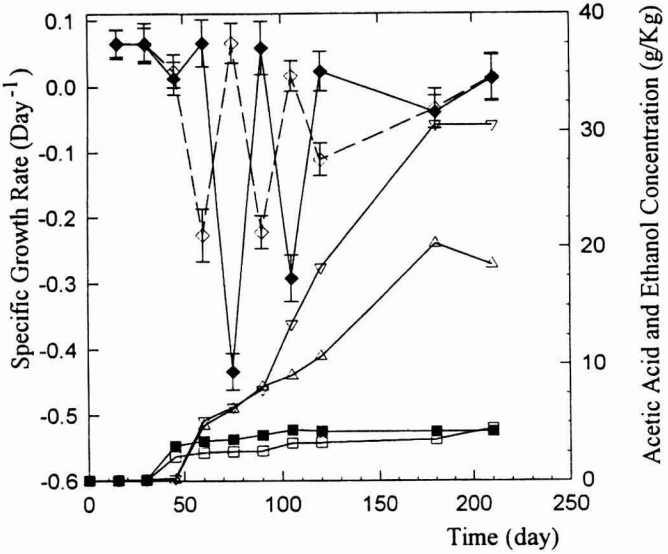

FIG. 3. EVOLUTION OF YEAST SPECIFIC GROWTH RATE, OF THE ACETIC ACID AND THE ETHANOL PRODUCT ON TOMATO PASTE STORED AT 10C
(◆) yeast specific growth rate (top), (◇) yeast specific growth rate (bottom), (∇) ethanol production (top), (Δ) ethanol production (bottom), (■) acetic acid production (top), (□) acetic acid production (bottom).

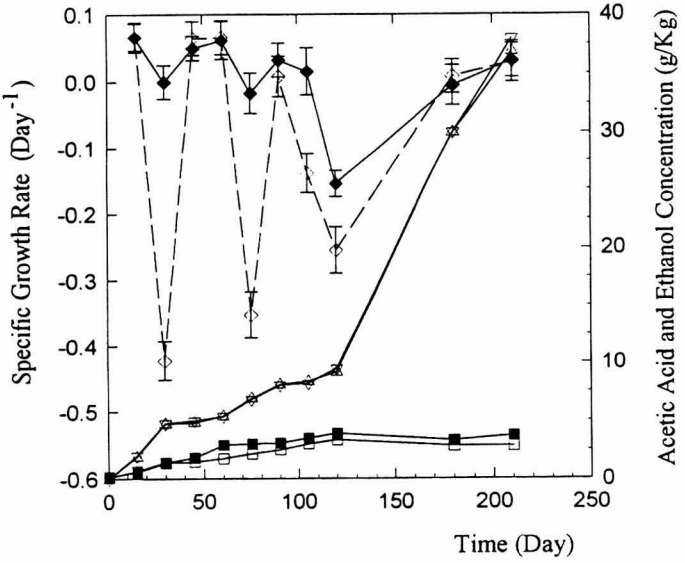

FIG. 4. EVOLUTION OF YEAST SPECIFIC GROWTH RATE, OF THE ACETIC ACID AND THE ETHANOL PRODUCT ON TOMATO PASTE STORED AT 25C
(◆) yeast specific growth rate (top), (◇) yeast specific growth rate (bottom), (∇) ethanol production (top), (Δ) ethanol production (bottom), (■) acetic acid production (top), (□) acetic acid production (bottom).

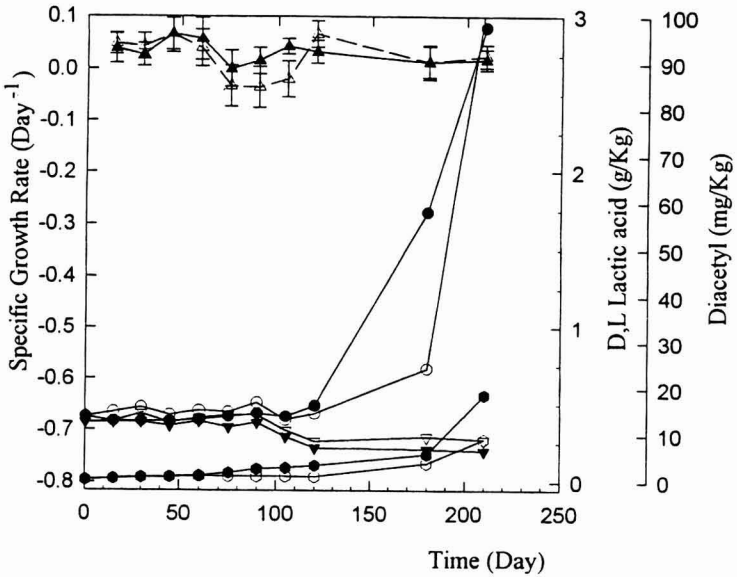

FIG. 5. EVOLUTION OF LACTIC ACID BACTERIA SPECIFIC GROWTH RATE, OF THE D-L LACTIC ACIDS AND DIACETYL PRODUCT ON TOMATO PASTE STORED AT 4C (▲) lactic acid bacteria (top), (Δ) lactic acid bacteria (bottom), (●) L-lactic acid (top), (○) L-lactic acid (bottom), (▼) D-lactic acid (top), (▽) D-lactic acid (bottom), (◉) diacetyl (top), (◌) diacetyl (bottom).

growth velocity specific for lactic acid bacteria found in stored samples at different temperatures and seems to be correlated with the different storage temperature conditions. In fact, with the sample stored at 25C, the inhibition (Fig. 7) was present in the top and bottom zones at about 30 days with the sugars quotient at 30% and about 40%, respectively. At 10C the inhibition (Fig. 6) occurred in the top area at about 90 days. This corresponds to a sugars quotient which was the same as that found in the upper product at 25C (26.2% in Table 3). The same behavior was found in the lower area of the drum where the sugars quotient was 40.9 (in Table 3). For the product stored at 4C (Fig. 5) the absence of a marked inhibition of lactic acid bacterial growth seems to be linked to the finding that the sugars quotient was always above 40% during storage. This suggests different lactic acid bacteria behavior linked both to the different aerobic conditions probably occurring in the product and to the sugar availability within the product. Since the sugar content remained high (sugars quotient $> 40\%$) growth inhibition did not occur. Conversely, when this value neared 40%, the growth of lactic acid bacteria subjected to low oxygen levels (bottom samples) was inhibited, whereas the same occurred for the upper samples with much lower sugars quotient (sugars quotient $< 30\%$). This last finding is in agreement with data reported by Kandler and Weiss (1986). They

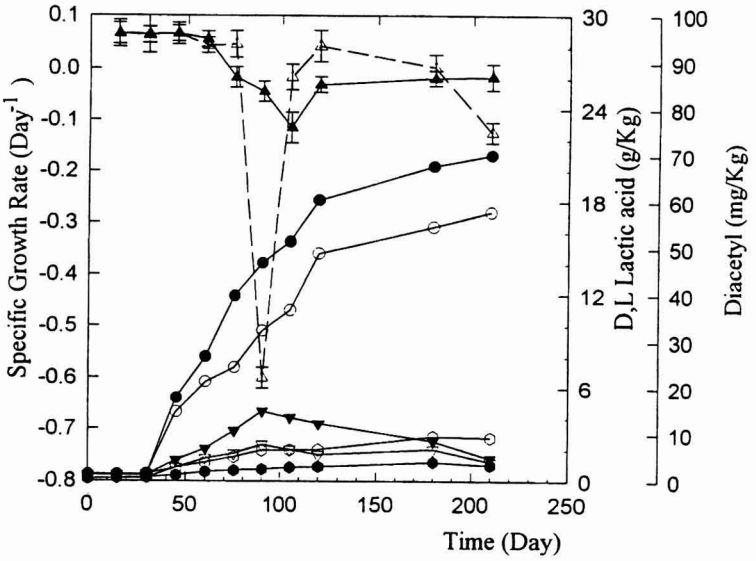

FIG. 6. EVOLUTION OF LACTIC ACID BACTERIA SPECIFIC GROWTH RATE, OF THE D-L LACTIC ACIDS AND DIACETYL PRODUCT ON TOMATO PASTE STORED AT 10C (▲) lactic acid bacteria (top), (Δ) lactic acid bacteria (bottom), (●) L-lactic acid (top), (○) L-lactic acid (bottom), (▼) D-lactic acid (top), (▽) D-lactic acid (bottom), (●) diacetyl (top), (○) diacetyl (bottom).

found that in aerobic conditions many microbes can reoxidize $NADH_2$, using oxygen as an electron acceptor, so that acetyl-CoA is not completely reduced to EtOH. Under these conditions more ATP is produced and may be used by microbes for growth. Regarding the production of different metabolites, the data concerning acetic acid (Figs. 2–4), D-lactic acid, L-lactic acid and diacetyl (Fig. 5–7) are reported. The analysis of variance revealed that for the samples taken at the two heights (top and bottom) the analytical compositional findings were significantly different, while no significant difference was seen in the samples taken at the same height (horizontal variance) but at different sites.

There was an upward trend in acetic acid growth at all storage temperatures. However, as to be expected the content of acetic acid differed notably with the different storage temperatures. At 4C (Fig. 2) there was almost the same acetic acid production at the top and at the bottom areas, although it was slightly higher in the top area of the drum.

At 10C (Fig. 3) the greatest production of acetic acid occurred in the top part of the drum. At 25C we saw (Fig. 4) an acetic acid production that was very fast. After 15 days it reached 0.6–0.7 g/kg in both the top and bottom

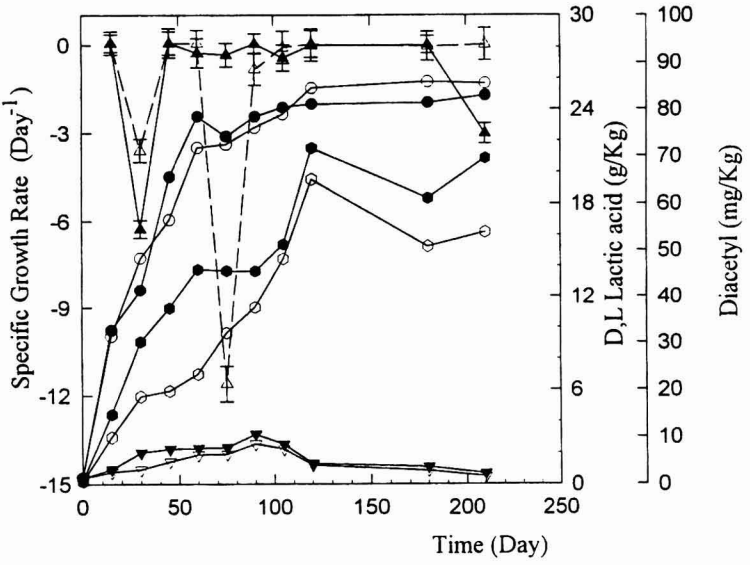

FIG. 7. EVOLUTION OF LACTIC ACID BACTERIA SPECIFIC GROWTH RATE, OF THE D-L LACTIC ACIDS AND DIACETYL PRODUCT ON TOMATO PASTE STORED AT 25C (▲) lactic acid bacteria (top), (Δ) lactic acid bacteria (bottom), (●) L-lactic acid (top), (○) L-lactic acid (bottom), (▼) D-lactic acid (top), (∇) D-lactic acid (bottom), (◉) diacetyl (top), (⊂) diacetyl (bottom).

samples. At this temperature, as well, the production of acetic acid was greater in the top area of the drum.

Regarding the diacetyl content, our findings may be summarized as follows: at storage temperatures of 4C (Fig. 5) and 25C (Fig. 7) there was a higher production of diacetyl in the top part of the drum, while at 10C (Fig. 6) there was a greater production of this metabolite in the bottom sampling zone (about 9.5 mg/kg produced after 210 days versus 3.5 mg/kg produced in the top area). It could be that the high level of acetic acid produced at 10C in anaerobic conditions (about 3.60 g/kg), which was considerably higher than what was produced at 4C (about 0.77 g/kg, see Fig. 2) and at 25C (about 2.80 g/kg, see Fig. 4), determines negative feed back on Pyruvic acid -- > -- > -- Acetate pathway, forcing D,L-lactic acid and diacetyl production. This may be confirmed (a) experimentally, by the greater production of D-lactic acid produced at 10C compared to that at 4C and at 25C, (b) by reports of Kandler (1983), showing that the diacetyl production is low when hexoses only are present in the medium but it increases when pyruvate is produced. At 4C L-lactic acid content (Fig. 5) is at the same concentration in the top and bottom areas of the drum (mean value about 0.4g/kg for the first 120 storage days).

At 10C the L-lactic acid production was decisively greater, going from about 0.4 g/kg to 18 g/kg (in the top part) and to about 15 g/kg (in the bottom part) after 120 days. The lowest lactic acid content in the bottom area of the drum may be correlated with the data in Fig. 6, showing an inhibition time of lactic acid bacteria activity briefer than in the top part of the drum, thus conditioning the final L-lactic acid level. In addition, at 25C, where the inhibition of lactic acid bacteria was the same in both sampling areas, the L-lactic acid production is the same at all storage times. As far as D-Lactic acid is concerned, at 4C its levels were found constant during the storage at about 0.3 g/kg (Fig. 5). At 10C, in the top part of the drum, there was a maximum production at about 90 days (4.5 g/kg) which then declined to 1.5 g/kg at 210 days. Finally, at 25C (Fig.7) a maximum was reached at about 90 days (3.18 g/kg) and then declined to 0.71 g/kg at 210 days. The same behavior is seen in the bottom area of the drum as reported in Fig. 5–7. These findings appear to be consistent with the presence of an enzyme able to break down D-lactic acid alone and seem to be in agreement with the literature (Kandler 1983) regarding NAD-independent lactate dehydrogenase (LDH) responsible for lactate oxidation.

The overall picture shows, however, that it is the L-lactic acid metabolite that has the greatest effect on the total lactic acid content, as widely reported in the literature (Luster 1978; Juven and Weisslowicz 1981; Vicini *et al.* 1988; Gherardi *et al.* 1988).

Metabolites in double tomato concentrate at different storage temperatures influenced taste and smell: fermented and sour taste was dependent on storage temperature and on the contamination level as well as on the different conditions occurring inside the drum. In fact, the sample stored at 4C (top section) had a sour taste and slight acid aroma after 75 days, while a sample taken from the bottom of the same drum had the same characteristics after about 120 storage days.

At 10C a persistent acid and/or fermented taste was found after about 45 days in both the top and bottom sections. Finally, in those samples preserved at 25C, taste and smell was greatly altered (high acidity) already after 15 days of storage.

Analytical-Compositional Aspects During Storage

The influence of storage temperature on analytical parameters is shown in Tables 2, 3 and 4.

Soluble Solids, Total Acidity and pH Evolution. It is interesting to note that the soluble solids in the bottom region of the drum remained stable for the whole storage period at 4C, while in the top section of the drum the soluble

TABLE 2.
CHANGES IN ANALYTICAL PARAMETERS OF TOMATO PASTE (≈ 30° Brix) STORED AT 4C

Storage Time (Day)	Sampling Zone	Soluble solids %	Total solids g %	pH	Volatile Acidity [c] g%	Total Acidity [d] g%	Fru/Glu ratio	Fru+Glu sum [e]	Total Sugar [f] g%	Sugar Quotient [g] %
0		30.5	31.8	4.36	0.013	2.17	1.08	15.44	16.7	48.6
15		30.4	31.8	4.37	0.017	2.16	1.08	15.33	16.7	48.3
30		30.1	31.8	4.39	0.019	2.18	1.09	15.04	16.6	47.3
45	T	30.1	31.8	4.33	0.022	2.23	1.11	14.89	16.6	46.7
60	O	30.1	31.4	4.31	0.024	2.22	1.16	15.01	16.6	47.9
75	P [a]	30.0	31.4	4.30	0.025	2.27	1.14	14.59	15.7	46.5
90		29.9	31.3	4.29	0.031	2.31	1.15	13.86	14.5	44.3
105		29.2	30.8	4.33	0.031	2.27	1.18	13.15	14.3	42.7
120		28.9	30.3	4.32	0.031	2.24	1.25	12.90	14.1	42.6
180		27.1	28.5	4.33	0.052	2.11	1.29	12.69	13.1	44.6
210		25.0	26.3	4.34	0.075	2.10	1.20	11.66	11.9	44.4
0		30.5	31.8	4.37	0.015	2.17	1.08	15.48	16.6	48.7
15		30.5	31.6	4.37	0.018	2.19	1.13	15.03	16.6	47.5
30	B	30.2	31.5	4.38	0.024	2.21	1.10	14.54	16.5	46.1
45	O	30.1	31.5	4.29	0.024	2.25	1.10	14.49	16.5	46.1
60	T	30.0	31.9	4.30	0.023	2.23	1.10	14.44	16.4	45.3
75	T	30.0	31.8	4.30	0.023	2.26	1.14	14.58	16.3	45.8
90	O	29.7	31.8	4.30	0.026	2.29	1.11	14.49	16.1	45.5
105	M [b]	30.4	31.8	4.29	0.025	2.10	1.18	14.07	15.9	43.9
120		30.5	32.1	4.30	0.024	2.10	1.29	13.94	15.9	43.4
180		30.4	31.8	4.34	0.024	2.10	1.11	14.43	15.8	45.4
210		29.8	30.9	4.34	0.024	2.10	1.08	14.07	15.3	45.5

[a] The sample was taken at 10 cm from the drum top. [b] The sample was taken at 15 cm from the drum bottom. [c] Volatile acidity expressed as acetic acid. [d] Total acidity (pH 8.1) expressed as citric acid monohydrate. [e] Sum of fructose and glucose by HPLC. [f] Total sugars determined by Fehling method. [g] Sugar quotient = ((Fru+Glu)/total solids) x 100.

solids remained stable only for the first 90 days and then declined until 25° Brix at the end of storage. At 10C the same thing occurred (Table 3). The soluble solids stayed stable in the bottom of the drum for about 60 days while in the top area for 30 days.

Finally, at 25C the soluble solids in both areas of the drum stayed the same for only 15 days, to confirm that at this temperature the compositional changes are very fast. This is even more apparent given the data in Tables 2, 3 and 4 regarding the total acidity trends at the different storage times. In fact, at 4C for both region samples, the total acidity remained substantially the same while at 10C it increased from 2.17 to 3.85 (top sample) and from 2.17 to 3.51 g% (bottom sample) at the end of the storage. This effect was even greater at 25C where after only 15 days a mean total acidity exceeding 3.0 g% was reached.

TABLE 3.
CHANGES IN ANALYTICAL PARAMETERS OF TOMATO PASTE
(≈ 30° Brix) STORED AT 10C

Storage Time (Day)	Sampling Zone	Soluble solids %	Total solids g %	pH	Volatile Acidity [c] g%	Total Acidity [d] g%	Fru/Glu ratio	Fru+Glu sum [e]	Total Sugar [f] g%	Sugar Quotient [g] %
0		30.5	32.6	4.36	0.013	2.17	1.08	15.66	16.6	48.0
15		30.3	32.2	4.39	0.015	2.17	1.09	15.28	16.6	47.4
30		30.2	32.1	4.45	0.016	2.17	1.05	15.02	16.3	46.9
45	T	26.5	28.8	4.32	0.062	2.52	0.99	11.31	12.0	39.3
60	O	24.8	26.0	4.03	0.180	3.04	1.16	5.99	7.0	23.1
75	P [a]	22.5	23.8	4.00	0.213	3.12	1.28	5.06	5.8	21.3
90		20.1	21.4	3.90	0.270	3.35	1.34	5.62	5.7	26.2
105		20.3	21.8	3.96	0.321	3.43	1.25	3.00	3.4	13.8
120		20.5	21.3	3.96	0.340	3.67	1.24	3.02	3.0	14.2
180		19.8	21.1	3.99	0.296	3.85	1.07	2.20	2.7	10.5
210		19.7	20.7	3.99	0.257	3.85	1.04	1.92	1.8	9.3
0		30.5	32.6	4.33	0.015	2.17	1.08	15.31	16.9	48.3
15		30.1	31.8	4.34	0.021	2.29	1.11	14.90	16.7	46.8
30	B	30.0	31.8	4.38	0.027	2.29	1.12	14.40	16.6	45.2
45	O	29.5	31.1	4.23	0.039	2.29	1.11	13.86	15.2	44.5
60	T	28.9	30.5	4.11	0.062	2.34	1.16	13.10	14.5	43.0
75	T	27.1	28.1	4.08	0.079	2.55	1.23	11.49	12.1	40.9
90	O	26.3	27.7	4.07	0.091	2.73	1.27	10.25	10.7	37.1
105	M [b]	25.9	27.1	4.04	0.115	3.03	1.26	8.57	8.4	31.6
120		25.8	27.8	4.01	0.122	3.11	1.27	8.40	8.6	30.2
180		25.6	27.6	4.02	0.235	3.32	1.19	8.28	8.5	30.0
210		25.5	27.5	4.01	0.241	3.51	1.12	8.01	8.4	29.1

[a] The sample was taken at 10 cm from the drum top. [b] The sample was taken at 15 cm from the drum bottom. [c] Volatile acidity expressed as acetic acid. [d] Total acidity (pH 8.1) expressed as citric acid monohydrate. [e] Sum of fructose and glucose by HPLC. [f] Total sugars determined by Fehling method. [g] Sugar quotient = ((Fru+Glu)/total solids) x 100.

Finally, as shown in Tables (2–4), pH decreased notably at 25C and 10C while it remained stable in the product stored at 4C.

Sugars and Organic Acids Evolution. The finding about sugar and organic acid use by the microbial flora may be summarized as follows. The FRU/GLU ratio increases considerably with the storage time of 25C both in top and bottom areas of the drum. Instead, this behavior is less evident in the storage samples at 4 and 10C. It is interesting to note that the FRU/GLU ratio at the higher storage temperatures increased during the first phase and thereafter decreased. These findings therefore show a differentiated use of fructose and glucose during the storage period, characterizing a preferred glucose use in the early storage period (increased FRU/GLU ratio) followed by a greater fructose use, compared to glucose in the late storage period (decrease in the FRU/GLU ratio).

TABLE 4.
CHANGES IN ANALYTICAL PARAMETERS OF TOMATO PASTE
(≈ 30° Brix) STORED AT 25C

Storage Time (Day)	Sampling Zone	Soluble solids %	Total solids g %	pH	Volatile Acidity [c] g%	Total Acidity [d] g%	Fru/Glu ratio	Fru+Glu sum [e]	Total Sugar [f] g%	Sugar Quotient [g] %
0		30.5	31.5	4.38	0.018	2.17	1.05	15.21	15.4	48.6
15		29.3	31.1	4.12	0.093	3.02	1.16	11.37	14.0	36.6
30		25.3	26.4	4.02	0.141	3.67	1.27	7.94	8.7	30.1
45	T	25.5	26.1	4.02	0.227	3.71	1.49	5.59	6.7	21.5
60	O	24.0	25.6	4.02	0.301	4.02	1.57	4.11	4.9	16.1
75	P [a]	22.9	24.1	4.00	0.345	4.12	1.47	3.71	4.1	15.4
90		22.2	23.7	3.96	0.379	4.21	1.37	3.49	4.4	14.8
105		22.4	23.5	3.94	0.378	4.21	1.30	3.46	4.3	14.7
120		22.0	23.2	3.95	0.375	4.16	1.23	3.08	4.1	13.3
180		21.1	22.3	3.96	0.280	4.02	0.72	1.95	2.8	8.8
210		21.0	22.2	3.97	0.199	4.01	0.70	1.91	2.7	8.6
0		30.4	31.5	4.38	0.018	2.17	1.03	15.49	16.1	48.5
15		29.0	30.2	4.22	0.049	2.67	1.05	13.63	15.2	45.2
30	B	27.3	29.2	4.12	0.078	2.81	1.13	11.45	11.7	39.3
45	O	25.8	27.2	4.11	0.176	3.57	1.29	7.34	7.8	27.0
60	T	24.0	24.8	4.09	0.270	4.11	1.98	4.09	5.8	16.5
75	T	23.1	24.7	3.97	0.273	4.11	1.82	3.79	4.9	15.4
90	O	23.5	24.6	3.96	0.290	4.13	1.57	3.63	4.4	14.8
105	M [b]	23.2	24.5	3.97	0.288	4.15	1.55	3.67	4.4	15.0
120		23.3	24.8	3.99	0.294	4.09	1.56	3.93	4.7	15.9
180		24.0	24.4	3.99	0.191	4.06	1.45	4.09	4.4	16.7
210		23.0	24.1	3.99	0.182	4.02	1.41	3.44	3.7	14.3

[a] The sample was taken at 10 cm from the drum top. [b] The sample was taken at 15 cm from the drum bottom. [c] Volatile acidity expressed as acetic acid. [d] Total acidity (pH 8.1) expressed as citric acid monohydrate. [e] Sum of fructose and glucose by HPLC. [f] Total sugars determined by Fehling method. [g] Sugar quotient = ((Fru+Glu)/total solids) x 100.

Citric acid showed an increased percentage breakdown with storage temperature. Its concentration was 19.1 g/kg at beginning of the storage but after 210 days it became 10% lower at 4C, 28% lower at 10C and 37% lower at 25C.

Malic acid behaved like citric acid, from a starting concentration of 1.95 g/kg, a percentage drop of about 14% at 4C and about 52% at 10C and about 60% at 25C was observed after 210 days of storage.

D-isocitric acid did not seem to be very much degraded at 4C, but at 10C and at 25C, after 210 storage days, the breakdown was about 26% and 28%, respectively, from a starting concentration of 0.45 g/kg.

Finally, for succinic acid, we found substantial unexplainable stability at 25C, against a degradation of about 25% both at 4C and at 10C after 210 storage days.

Color and HMF Evolution. The last aspect of this study regards the influence of storage temperature on product color (unreported data) and on the evolution of hydroxymethylfufurol (HMF) content. The findings show a substantial color stability (the a/b ratio ranged from 2.32–2.15 while the L parameter ranged from 24.2–25.0). The HMF content was almost constant at about 5 mg/kg at all temperatures.

CONCLUSION

The analytical compositional variance of double tomato concentrate stored in nonsterile drums was determined. Our findings show that a practical recommendation would be to store the product at temperatures below or equal to 4C. At that temperature the product remained relatively stable from an organoleptic and compositional standpoint for at least three months. The analytical data have in fact confirmed at this temperature a substantial consistency of main analytical parameters. Soluble solids, the parameter usually used in the trade of this semifinished product, stayed constant for at least 90 days in both the top and bottom of the drum. Citric acid, the main tomato acid also remained stable at 4C. The decay was in fact about 10% in 210 days of storage. From a purely technological outlook, a slower product decay may be obtained by preventively cooling the semifinished tomato to less than 10C immediately after concentration, instead of placing the product in drums at 65–70C and then letting it cool. In such a way prolonged storage at temperatures ideal for lactic acid bacteria growth is avoided before the ideal storage temperature is reached. In fact, our data show that samples slowly cooled and stored at 10C and 25C had a remarkable production of D,L-lactic acid exceeding 1 g/kg of product in the first days of storage. These values are decisively high compared to the generally accepted quality standard (D,L-lactic acid < 0.5 g/kg) for a good quality product.

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TWIN-SCREW EXTRUSION OF RICE FLOUR: EFFECT OF EXTRUDER LENGTH-TO-DIAMETER RATIO AND BARREL TEMPERATURE ON EXTRUSION PARAMETERS AND PRODUCT CHARACTERISTICS

SUVENDU BHATTACHARYA and GOUR S. CHOUDHURY

Fishery Industrial Technology Center
School of Fisheries and Ocean Sciences
University Alaska Fairbanks
900 Trident Way
Kodiak, AK 99615

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ABSTRACT

The effects of extruder length-to-diameter (L/D) ratio and barrel temperature on extrusion system parameters and extrudate characteristics during twin-screw extrusion of rice flour were studied. The L/D ratio and barrel temperature were varied between 16–24 and 75–185C, respectively. The extrusion system parameters studied were thrust bearing pressure and specific mechanical energy. The expansion ratio, bulk density and maximum force of the extruded product, as affected by the L/D ratio and barrel temperature, were investigated. Experimental data on system parameters and extrudate characteristics were fitted to a second degree polynomial.

*The linear as well as the quadratic effect of L/D ratio, and the quadratic effect of temperature were significant ($p \leq 0.10$) in most cases. The significant ($p \leq 0.10$) interaction term (L/D ratio * temperature) for most of the response functions revealed that a change in the L/D ratio can affect the influence of temperature on system parameters and extrudate attributes. The hardness of the extrudates increased with increasing L/D ratio. Microstructure of the extrudates suggested that a barrel temperature above 150C is desirable to get an expanded product.*

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INTRODUCTION

Extrusion processing has developed rapidly during the last decade, and can now be considered as a technology in its own right (Cheftel 1990). A myriad of fabricated, cooked, flavored and shaped products are manufactured using food extruders. Cooking extruders are used to produce breakfast cereals, noodles and pasta, biscuits, snack foods, confectionery, chewing gum, modified starches, dried soups, pet foods, crackers, bread crumbs, dried beverage powders, and texturized proteins (Hauck 1980). The twin-screw extruders, developed in recent years, are increasingly used to process starch-based food products. Although the twin-screw extruders are more complex and costly than their conventional single-screw counterparts, they possess several advantages. Better control of product characteristics can be achieved by monitoring the residence time and internal shear of heat-sensitive food ingredients. In addition, twin-screw extruders are operational at very low moisture requiring no or minimum postextrusion drying (Dziezak 1989; Harper 1989).

Extensive studies on extrusion processing of cereals, particularly corn and wheat, to generate ready-to-eat (RTE) breakfast cereals and snacks, have been carried out (Colonna *et al.* 1989; Hsieh *et al.* 1990). Literature on thermoplastic extrusion of rice is rather sparse. Some studies have been reported in recent years (Pan *et al.* 1992; Kim and Maga 1990; Kumagai *et al.* 1987). Fewer rice based extruded products are available compared to extruded corn and wheat products. Nevertheless, rice crackers and snacks are gaining popularity in European and American markets in recent years (Tuley 1992). The advantage of using rice in snacks and breakfast cereals lies in the fact that rice is nonallergenic, gluten-free, and naturally low in sodium; it is an important source of vitamins and minerals, and has about 7% high-quality protein, and only a trace of fat (Dziezak 1991). Rice products also impart a fatty mouthfeel and texture and are used in developing foods with low fat content (Anon. 1991).

To develop a rice based extruded product, it is essential to determine the effects of the extrusion variables on the system parameters and product characteristics. The knowledge of system parameters, such as pressure and mechanical energy during extrusion is important for process operation and control. The effect of length-to-diameter (L/D) ratio on product and extrusion characteristics has not been studied extensively. Kitabatake *et al.* (1985) and Bhattacharya *et al.* (1992) showed that the length of the extruder, or the length-to-diameter (L/D) ratio, is an important factor in relation to texture of the developed product and on the rheology of the dough during extrusion.

The objective of our study was to determine the effect of extruder L/D ratio and barrel temperature on the system parameters and product characteristics during twin-screw extrusion of rice flour.

MATERIALS AND METHODS

Material

Milled flour of California medium grain rice was obtained from Pacific Grain Products, Inc., Woodland, CA. The proximate composition of rice flour, determined following the AOAC (1984) methods is given in Table 1. The particle size distribution of rice flour, as measured by the sieve analysis, is shown in Table 2.

TABLE 1.
PROXIMATE COMPOSITION OF RICE FLOUR

Components	Mean ± SD
Moisture (%)	9.95 ± 0.08
Protein (%)[1]	5.85 ± 0.09
Fat (%)	0.56 ± 0.06
Ash (%)	0.47 ± 0.05
Crude fiber (%)	1.74 ± 0.07
Carbohydrate (%)[2]	81.43

[1]Protein = N*5.95, [2]Carbohydrate by difference

TABLE 2.
PARTICLE SIZE DISTRIBUTION OF RICE FLOUR

	US sieve number	%
Retained on	50	0.9
	80	16.1
	100	7.5
	140	19.0
	200	18.5
Passed through	200	38.0

Extruder

All experiments were carried out using a co-rotating Clextral twin-screw extruder (Model BC 21, Clextral S.A., Firminy Cedex, France) designed with modular 100 mm barrels, and bored with two 25 mm diameter holes. The extruder is equipped with a twin-screw (30 mm pitch) metering feeder (K-Tron Corporation, New Jersey), and can impose a wide range of temperatures, pressures, and shear upon the material being processed. Screw speed, screw geometry and balance of energy to the product can be varied. The unit is equipped with electronic resistance heaters and digital indicators for main drive, cutter and feeder speeds, thrust bearing pressure (backward pressure offered by food during extrusion), barrel temperature, and die or melt temperature (temperature of the food material at the die) and pressure. A die drilled with four tapered holes of 2 mm diameter was used in this study. The length-to-diameter (L/D) ratio of the extruder was varied at 16, 20 and 24 corresponding to total extruder length of 400, 500 and 600 mm, respectively. The details of the screw configuration are shown in Table 3. The extruder screw elements were all double-start and forward pitched.

TABLE 3.
SCREW CONFIGURATION OF THE EXTRUDER AT DIFFERENT L/D RATIOS

Screw Element		Number of element for L/D ratios of		
Length (mm)	Pitch (mm)	16	20	24
50[F]	50	1	1	2
50	33.3	1	2	3
50	25	1	2	2
25	25	2	2	2
50	16.6	2	2	2
25[D]	16.6	4	4	4

[F]Feed end of the extruder [D]Die end of the extruder

The extrusion temperature used throughout this paper refers to the temperature of the barrel surface in contact with food. The barrel temperature of the last 100 mm section at the die end was maintained at 75, 100, 125, 150, 175 or 185C for different experimental runs. The 100 mm section preceding to the last section (at die end) was maintained at 80C followed by a stepwise decrease of 20C for each 100 mm barrel section towards feed end subject to a

minimum temperature of 20C at the feed section. The screw speed was 100 rpm. The extruder was run with the feed for at least 5 min after attaining constant torque and pressure; the readings such as percent torque and thrust bearing pressure were then noted, and the extrudates were collected for further analysis.

Preparation of Feed for Extrusion

The moisture content of the extruder feed was adjusted to about 18%. The feed was packed in double-walled polyethylene bags and allowed to equilibrate overnight at about 5C in a refrigerator. Before extrusion, the feed was allowed to come to ambient temperature (about 21C) and mixed again after checking the moisture content.

Extrusion

The moistened rice flour was fed to the extruder using the twin-screw metering feeder. Water was added to the feed during extrusion at the feeding end through a stroke dosing pump (Model DKM-K20, Clextral S.A., Firminy Cedex, France) such that the final moisture content of the feed was maintained at 25%. The feed rate of the extruder at steady state condition was 6.4 kg/h. All extrusion trials were replicated once.

Drying of Extrudates

The cylindrical rods, obtained from the extruder, were dried (19 ± 1C) in shade for 24 h to attain a moisture content of about 8%.

Expansion Ratio

The expansion ratio (ER) of the dried extruded rods was measured as the ratio of the cross-sectional areas of the extruded rods to that of the die. The ER values were obtained from five random samples of four observations on each sample.

Bulk Density

The bulk density (ρ_B) of individual dry, cylindrical extrudates was estimated by determining the mass and volume of five samples. The volume of the cylindrical rod was calculated by determining the average diameter and length of the individual product by using a dial thickness gauge with a resolution of 0.01 mm.

Maximum Force

The maximum force (F_M) or hardness of dry extrudates was determined as the peak force offered by the sample during shearing in an Instron Universal Testing machine (Model No. 1000, Instron Corporation, Canton, MA) with a Warner-Bratzler attachment. The cross-head speed was 500 mm/min; the full load scale was either 100 N or 10 N. The stainless steel shear blade was 1.22 mm thick with a shear angle of 60°. The reported values are the averages of 5 determinations with two replications each.

Specific Mechanical Energy

The specific mechanical energy (SME) input to the ingredient was calculated using the equation (Hsieh *et al.* 1990):

$$SME = \frac{rpm\ (run)}{rpm\ (rated)} * \frac{\%\ Torque\ (run)}{100} * \frac{Motor\ power\ (rated)}{Feed\ rate} \qquad (1)$$

Scanning Electron Microscopy

The extrudates were frozen at −80C, fractured, and dried in a freeze dryer at −50C for 3 h. The samples were coated with gold-palladium in a sputter coater (SPI Supplies, West Chester, PA). The cross-sections of the extrudates were examined under scanning electron microscope (Model JSM 35, JEOL USA Inc., Peabody, MA) at a voltage of 20 kV.

Statistical Analysis

The extrusion process variables, L/D ratio (X_1) and extrusion temperatures (X_2), were related to the response function (Y_i) by a second degree polynomial (Eq. 2) using the least squares technique (Little and Hills 1978):

$$Y_i = b_o + \sum_{i=1}^{2} b_i X_i + \sum_{\substack{i=1 \\ i \le j}}^{2} \sum_{j=1}^{2} b_{ij} X_i X_j + \epsilon \qquad (2)$$

The coefficients of the second degree polynomial are b_o, b_i, and b_{ij} and ϵ is the random error.

The response functions were expansion ratio (ER), bulk density (ρ_B), maximum force (F_M), thrust bearing pressure (ΔP) and specific mechanical energy (SME). The nonsignificant ($p = 0.10$) terms in the polynomial, judged by F-test, were deleted and were recalculated to have the final polynomial. The significance of the multiple correlation coefficient (R) was judged at a probability level of 0.001.

RESULTS

Specific Mechanical Energy

The specific mechanical energy (SME), defined as the mechanical energy input to obtain 1 kg of extrudate, varied between 205 and 263 kJ/kg as shown in Fig. 1. The coefficients of the second order polynomial relating L/D ratio (X_1) and barrel temperature (X_2) to SME are shown in Table 4. The effect of

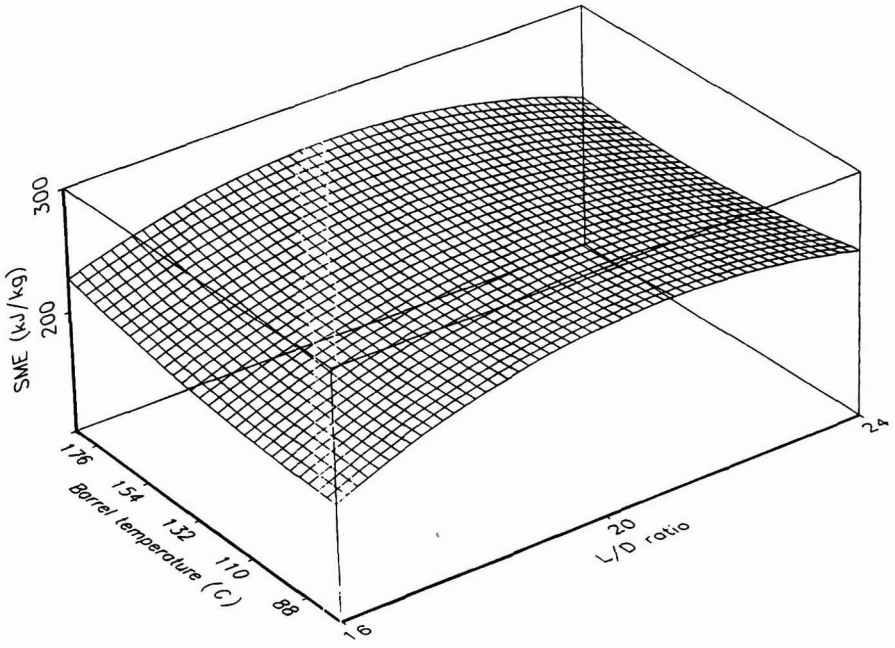

FIG. 1. SPECIFIC MECHANICAL ENERGY (SME) DURING EXTRUSION OF RICE FLOUR AT DIFFERENT LENGTH-TO-DIAMETER (L/D) RATIO AND BARREL TEMPERATURE

the L/D ratio on SME was complex. An increase in the L/D ratio from 16 to 20 at all temperatures increased SME. On the other hand, an increase of L/D ratio beyond 20 was associated with a slight decrease in SME (Fig 1). Increase in temperature increases SME at a low L/D ratio (such as 16) but is fairly constant at higher L/D ratios. The result of temperature effect at low L/D ratio agrees with the findings of Likimani *et al.* (1991) who observed an increase in specific power with an increase in barrel temperature while extruding a mixture of corn and soybean in presence of a thermostable enzyme with a L/D ratio of 20 and feed moisture of 17%. In a review, Wiedmann (1990) noted an opposite effect of barrel temperature on SME. A decrease in SME values was associated with an increase in barrel temperature from 80 to 190C during extrusion of wheat flour.

As shown in Table 4, only the linear effect of temperature was found to be nonsignificant at a probability level of 0.1. The significant ($p \leq 0.10$) interaction term (L/D ratio * temperature) indicates that a change in the L/D ratio can alter the influence of temperature on the SME during extrusion. The relationship of SME with L/D ratio (X_1) and barrel temperature (X_2), after removing the nonsignificant term, is given by:

$$SME = -795.83 + 101.44X_1 - 2.33X_1^2 - 4.42*10^{-2}X_1X_2 + 3.73*10^{-3}X_2^2 \quad (3)$$

Equation (3) accounted for 71.9% of the total variation in the SME values.

Thrust Bearing Pressure

The thrust bearing pressure (ΔP) is an indication of the backward pressure exerted by the food material on the screw root during extrusion (Harper 1981). In the present study, the ΔP values varied between 1.6 and 6.4 MPa as shown in Fig. 2. Increase in barrel temperature decreased the ΔP values. This result is in agreement with the findings of Arora *et al.* (1993), Wiedmann (1990) and Valle *et al.* (1987). The effect of the L/D ratio on ΔP was complex; at low barrel temperature, an increase in L/D ratio increased ΔP whereas at high temperatures the opposite effect was noted.

The coefficients of the regression equation for thrust bearing pressure (ΔP) are presented in Table 4. The linear effect of temperature was nonsignificant at a probability level of 0.1. This linear temperature term was removed and the recalculated equation (Eq. 4) accounted for 85.4% of the total variation in ΔP.

$$\Delta P = -18.25 + 2.47X_1 - 4.96*10^{-2}X_1^2 - 3.32*10^{-3}X_1X_2 + 1.03*10^{-4}X_2^2 \quad (4)$$

TABLE 4.
COEFFICIENTS OF THE REGRESSION EQUATION, MULTIPLE CORRELATION COEFFICIENT (R) AND ITS SIGNIFICANCE LEVEL (p) FOR DIFFERENT RESPONSE FUNCTIONS

Parameter	Regression coefficient for				
	Specific Mechanical Energy	Thrust Bearing Pressure	Expansion Ratio	Bulk Density	Maximum Force
b_0	-853.87	-18.64	5.16	2984.43	-382.49
b_1	104.43	2.49	-0.66 ^{NS}	-179.25	17.75
b_2	0.44 ^{NS}	$0.29*10^{-2}$ ^{NS}	$0.12*10^{-1}$ ^{NS}	6.49 ^{NS}	3.11
b_{11}	-2.37	$-0.50*10^{-1}$	$0.18*10^{-1}$	3.42	-0.25 ^{NS}
b_{12}	$-0.53*10^{-1}$	$-0.34*10^{-2}$	$-0.26*10^{-3}$ ^{NS}	0.29	$-0.25*10^{-1}$ ^{NS}
b_{22}	$0.28*10^{-2}$	$0.97*10^{-4}$	$0.15*10^{-3}$	$-0.78*10^{-1}$	$-0.10*10^{-1}$
R	0.85	0.92	0.96	0.97	0.93
p<	0.001	0.001	0.001	0.001	0.001

^{NS}Nonsignificant at p = 0.1

Expansion Ratio

The response surface for the expansion ratio (ER) is presented in Fig. 3. The L/D ratio had very little influence on product expansion. A meager increase in expansion ratio was observed with increasing L/D ratio. Product expansion increased markedly with increasing barrel temperature (Fig. 3). Similar observations of the effect of temperature on product expansion were reported for wild rice by Kim and Maga (1990). Pan *et al.* (1992) observed a maximum product expansion at 158 and 145C for waxy and nonwaxy rice, respectively. These researchers reported a decrease in ER values when the barrel temperature was raised above 160C. In our experimental temperature range (75–185C), such decreasing effect on the expansion ratio was not observed. Table 4 shows the coefficients of the second degree regression equation. Only the quadratic effects of both L/D ratio and barrel temperature were significant at a probability level of 0.1. The nonsignificant terms were removed and the recalculated equation (Eq. 5) shows the relationship of expansion ratio with L/D ratio (X_1) and barrel temperature (X_2):

$$ER = -0.49 + 8.91*10^{-4}X_1^2 + 1.73*10^{-4}X_2^2 \quad (5)$$

Equation (5) accounts for 91.0% of the total variation in the expansion ratio.

Bulk Density

Figure 4 shows the response surface for bulk density (ρ_B) as a function of L/D ratio and barrel temperature. Barrel temperature had a much greater effect on bulk density than did the L/D ratio. An increase in temperature markedly reduced the bulk density of the product and showed a curvilinear relationship (Fig. 4). The effect of L/D ratio was dependent upon temperature level. At a low barrel temperature, an increase in L/D ratio decreased bulk density but the trend was opposite at high temperatures.

Table 4 shows the coefficients of the regression equation for bulk density. The linear term for temperature was found nonsignificant at a probability level of 0.1. The resultant polynomial that accounted for a total of 92.9% variation in bulk density is given by:

$$\rho_B = 3852.65 - 223.92X_1 + 4.06X_1^2 + 0.43X_1X_2 - 6.39*10^{-2}X_2^2 \quad (6)$$

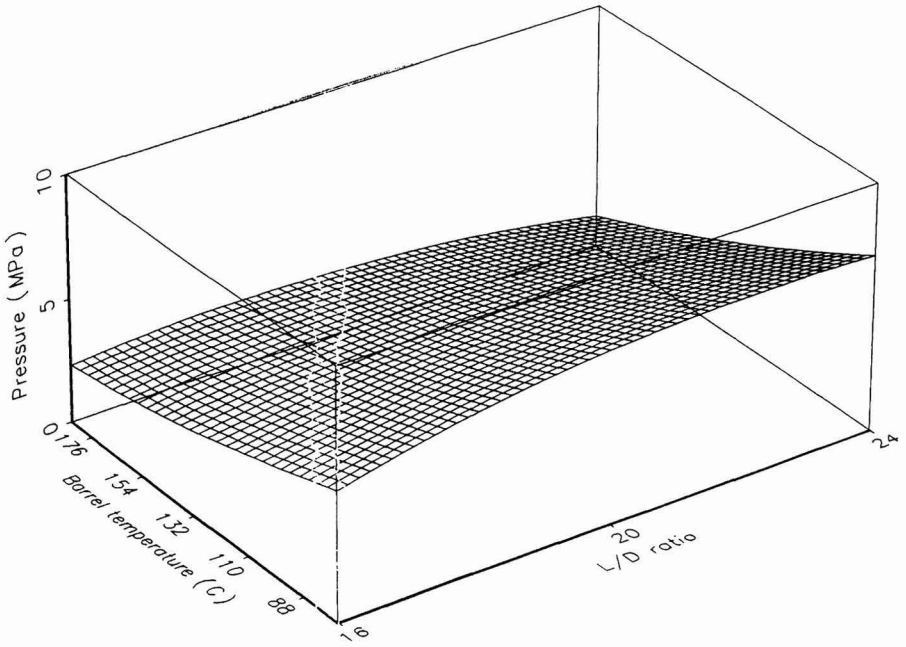

FIG. 2. THRUST BEARING PRESSURE (ΔP) DURING EXTRUSION OF RICE FLOUR AT DIFFERENT LENGTH-TO-DIAMETER (L/D) RATIO AND BARREL TEMPERATURE

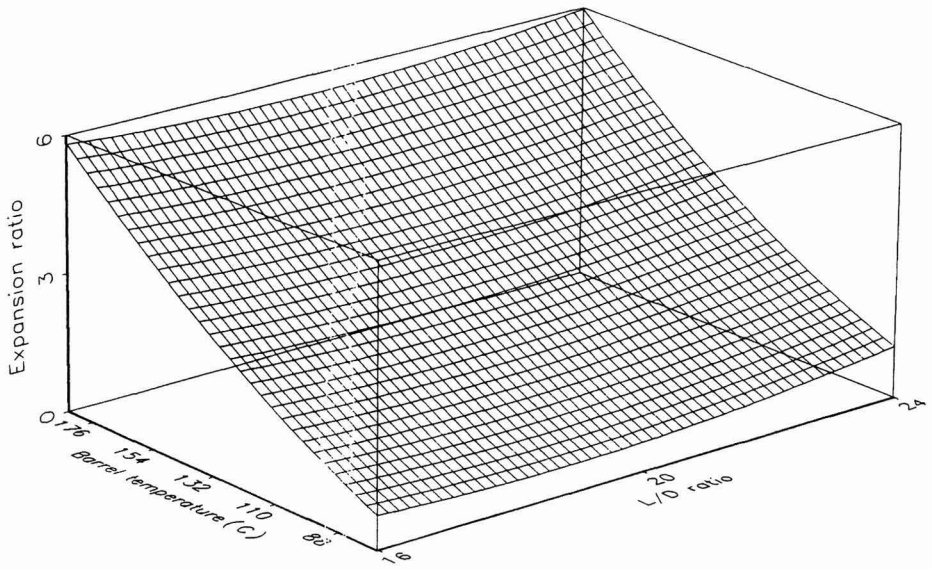

FIG. 3. EXPANSION RATIO OF THE DRY EXTRUDED RODS AT DIFFERENT LENGTH-TO-DIAMETER (L/D) RATIO AND BARREL TEMPERATURE

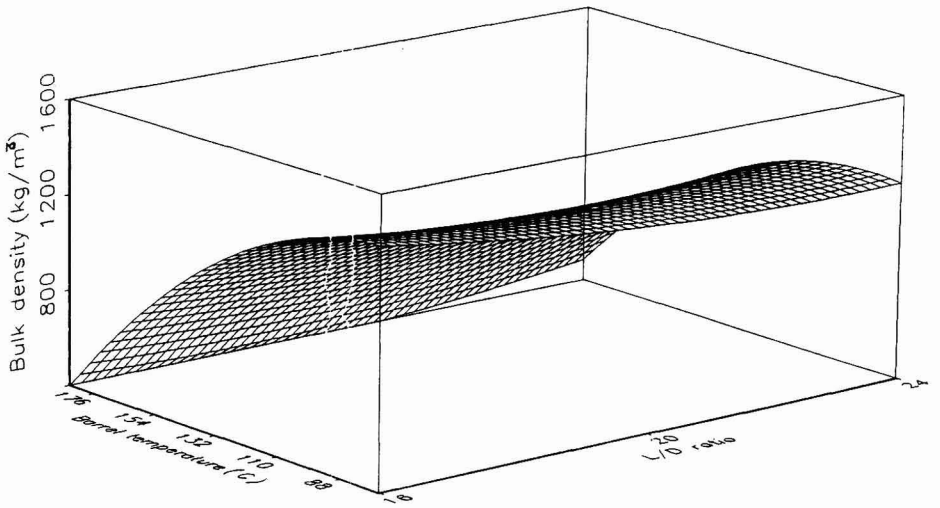

FIG. 4. BULK DENSITY OF THE DRY EXTRUDED RODS AT DIFFERENT LENGTH-TO-DIAMETER (L/D) RATIO AND BARREL TEMPERATURE

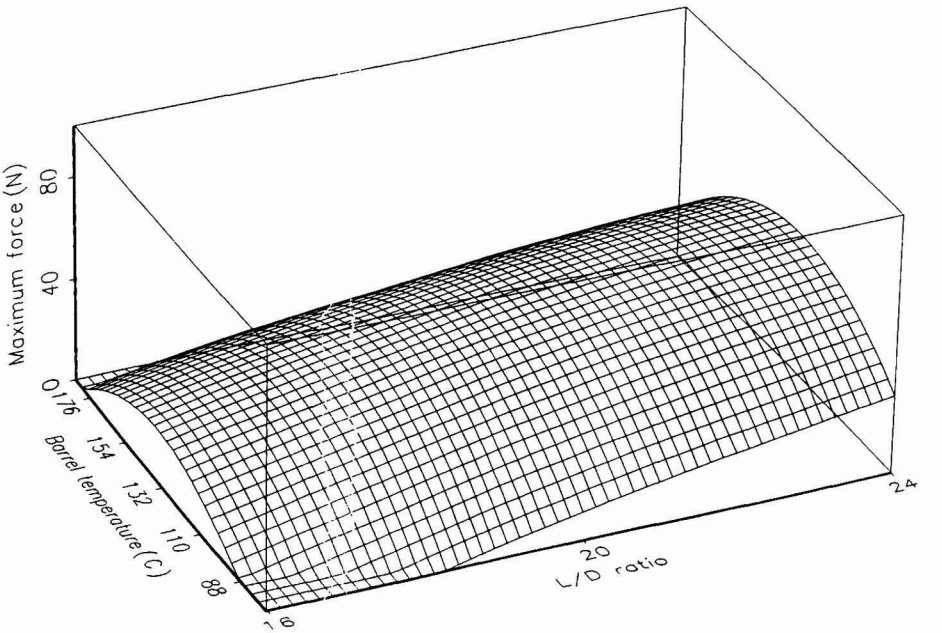

FIG. 5. MAXIMUM FORCE OF THE DRY EXTRUDED RODS AT DIFFERENT LENGTH-TO-DIAMETER (L/D) RATIO AND BARREL TEMPERATURE

Maximum Force

The maximum force (F_M) or hardness of dry extruded rods during shearing varied between 1.6 and 60.2 N, and the response surface for maximum force is shown in Fig. 5. Increase in L/D ratio markedly increased the FM values. This shows that extrusion with a high L/D ratio or long barrel generates a harder product. Increase in barrel temperature increased F_M except at higher temperatures where a decrease was noted. During extrusion of corn starch at a feed moisture of 23%, Owusu-Ansah *et al.* (1984) obtained similar results for the effect of temperature. Aboagye and Stanley (1987) also observed similar effect of temperature while extruding peanut flour at 35% moisture.

Table 4 shows the coefficients of the regression equation for maximum force. L/D ratio exerts a linear effect. The linear as well as the quadratic effect of barrel temperature was also significant ($p \leq 0.10$). The equation accounting for a total variation of 86.1% maximum force is given by:

$$F_M = -195.60 + 4.21X_1 + 2.31X_2 - 8.91*10^{-3}X_2^2 \quad (7)$$

Scanning Electron Microscopy

Figure 6 (A–F) shows photomicrographs of the samples extruded with a barrel temperature of 75–185C. Increase in barrel temperature enhanced the diameter of the extrudates. Low extrusion temperatures (75–150C) caused the extrudates to remain dense and laminated [Fig. 6 (A–D)]. However, progressive increase in temperatures resulted in vacuolation in the structure due to the formation of air cells and the surface appeared flaky and porous. Thus it is desirable to have a barrel temperature above 150C to obtain a highly expanded product. The formation of air cells started near the center, particularly at low temperatures (75–150C). At higher temperatures, these air cells were evenly distributed throughout the structure. The effect of the L/D ratio of the extruder on product microstructure was not clear and the micrographs show no apparent differences.

DISCUSSION

Temperature plays a significant role on extrusion and product characteristics. Apparent viscosity of the dough is affected by temperature or by L/D ratio; an increase in L/D ratio increases the residence time of the dough inside

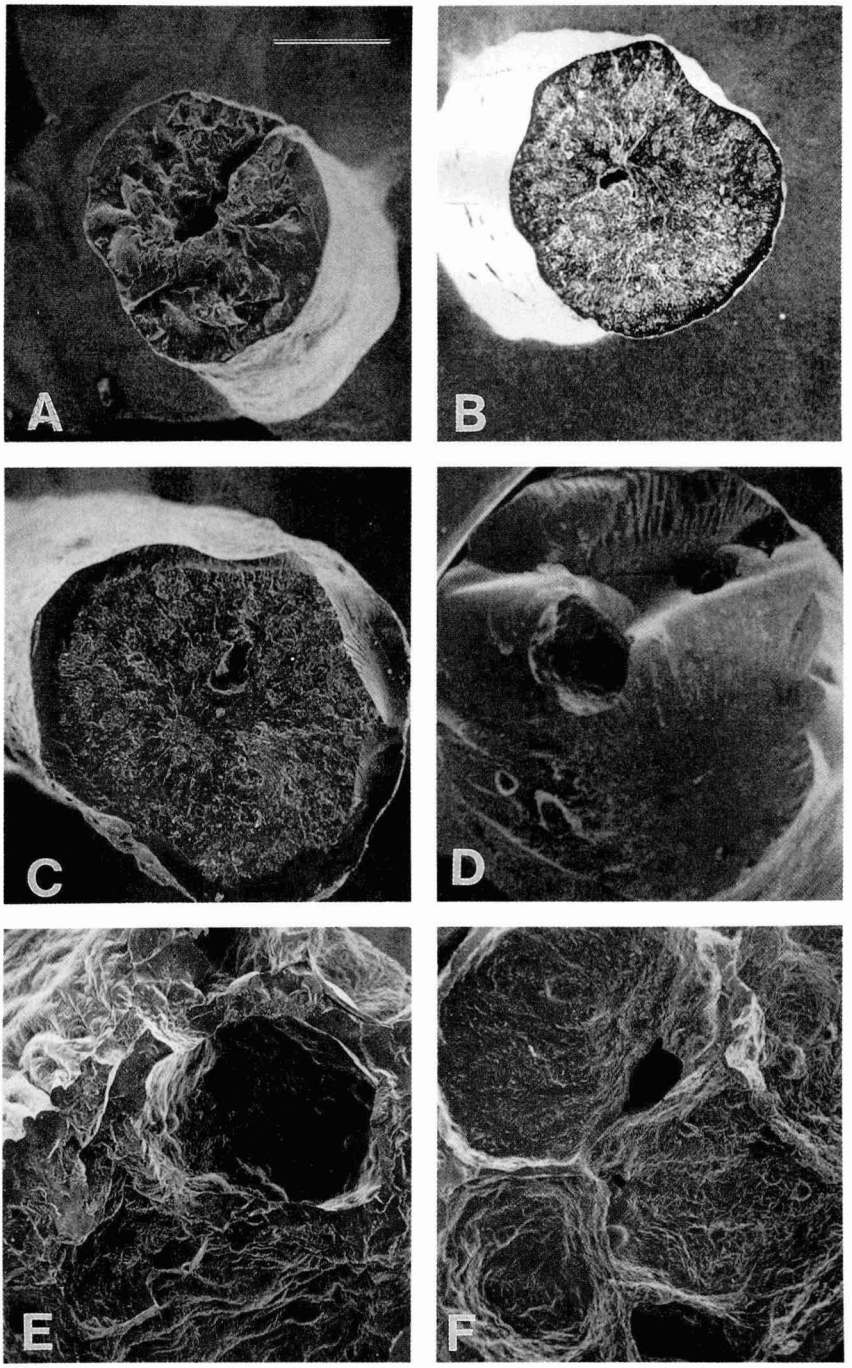

FIG. 6. SCANNING ELECTRON MICROGRAPHS OF THE CROSS-SECTIONS OF THE EXTRUDATE OBTAINED WITH AN L/D RATIO OF 20 AT BARREL TEMPERATURES OF: (A) 75, (B) 100, (C) 125, (D) 150, (E) 175, AND (F) 185C
Scale bar indicates 1000 μm in all micrographs.

the extruder. Harper *et al.* (1971) gave an empirical model (Eq. 8) for cooked cereal dough:

$$\eta = C_1 \dot{\gamma}^{C_2} e^{C_3/T} e^{C_4 M} \quad (8)$$

where η is the apparent viscosity, $\dot{\gamma}$ is the shear rate, T is temperature, M is moisture content, and C_1, C_2, C_3 and C_4 are constants. Levine (1983) stated that C_3 is positive. Thus, at a constant shear rate and moisture content, an increase in T decreases η. Besides, viscosity of rice flour dough is also affected by the extent of gelatinization. At a low temperature and/or short residence time (due to low L/D ratio), the gelatinization is incomplete and shows lower viscosity. The onset of gelatinization with increasing extrusion temperature or L/D ratio increases apparent viscosity. After completion of starch gelatinization, the apparent viscosity drops again with further increase in temperature according to Eq. (8). As a result, the torque decreases resulting to lower SME values. The pressure developed during extrusion also behaves in a similar fashion. As noted by Bhattacharya and Hanna (1987), the specific pressure (pressure per unit mass flow rate) is directly proportional to the apparent viscosity of the dough in the extruder.

An increase in barrel temperature possibly increased the extent of gelatinization and the content of superheated steam that caused the extrudate to expand more yielding a low density product. Starch gelatinization was incomplete at low L/D ratio and temperature (i.e., short residence time and insufficient thermal energy input). Hence, inadequate binding in the product resulted in low values for maximum force. With increase in L/D ratio and/or temperature, the extent of gelatinization increased that caused better binding as reflected by higher values for maximum force. Further, at high temperatures (160–185C), the extrudates were too dry ('crisp') and hence the maximum force decreased.

Microstructure of the extrudate revealed that low barrel temperature cannot develop the air cells due to insufficient gelatinization and binding leading to a collapsed structure as it comes out of the die at ambient pressure. At higher temperatures (175–185C), air cells with developed cell walls are present and gelatinized starch was able to prevent the collapse of the air cells.

CONCLUSIONS

The length-to-diameter (L/D) ratio of the extruder exerts a linear as well as quadratic effect on the extrusion system parameters (specific mechanical energy

and thrust bearing pressure) and product attributes (expansion ratio, bulk density and maximum force). Increase in L/D ratio yields a harder product. A L/D ratio of 16 or 20 is sufficient to generate a crunchy expanded product. The effect of barrel temperature on these parameters is usually complex and its quadratic effect is prominent. The temperature effect is dependent upon both the L/D ratio and the level of extrusion temperature. Scanning electron microscopy of the extrudates revealed that the barrel temperature should be above 150C to obtain an expanded product from rice flour.

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KINETICS OF THERMAL SOFTENING OF WHITE BEANS EVALUATED BY A SENSORY PANEL AND THE FMC TENDEROMETER

A. VAN LOEY, A. FRANSIS, M. HENDRICKX, G. MAESMANS AND P. TOBBACK

Katholieke Universiteit te Leuven
Faculty of Agricultural Sciences
Centre for Food Science and Technology
Unit Food Preservation

Kardinaal Mercierlaan 92
B-3001, Leuven, Belgium

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ABSTRACT

The temperature dependence of the rate index of thermal softening of white beans (Phaseolus vulgaris, *subsp.* nanus *Metz. variety* Manteca de Leon) *was determined within the temperature range of 90C to 122C using an industrial FMC tenderometer and a trained sensory panel. Based on sensorial results a first order reaction was assumed, whereas the tenderometer values were described by a biphasic model. Using a multiple linear regression analysis on the sensorial data, the activation energy for the heat induced firmness degradation of beans was calculated as 130.8 kJ/mole. Activation energies of both phases of the biphasic model were obtained by a two-step linear regression on the tenderometer data. Activation energies of both phases were equal, namely 105.1 kJ/mole.*

INTRODUCTION

Thermal processing of food results in an extended shelf-life but affects both the nutritional and the sensorial quality of the product. Kinetic data on the relevant quality attributes are necessary to optimize thermal processes. In a study by Schutz *et al.* (1974) consumers rated flavor and texture of vegetables as the

[1]Please address correspondence to author Hendrickx. Tel: 32-16-22.09.31, Fax: 32-16-29.38.05

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most important sensory attributes. In quality control and in research and development there is a pressing need for instrumentation that will enable rapid and easy measurement of flavor and quality attributes to bypass wherever possible the more expensive and time-consuming panel work (Sawyer 1971). Hereto the instrumental method should highly correlate with the sensory evaluation of the quality attribute.

The FMC pea tenderometer is a commercially important texture test instrument used to measure pea tenderness in the world's major pea areas and establish the price per unit weight payed to the grower (Voisey and Nonnecke 1973; Voisey and Kloek 1978). A continuing problem is standardization of the mechanical instrument. In the case of a pea tenderometer the means by which the instrument is standardized have been laid down and are directed towards proper maintenance of the mechanical components and checking the sensitivity of the force indicating system. Voisey and Nonnecke (1971, 1972) have shown that in spite of official inspections, these methods are not sufficiently adequate to ensure standardization of all tenderometers in a given region.

A review of kinetic studies on hardness degradation shows that the rate of softening, as measured by a physical test, follows first order kinetics (Suzuki *et al.* 1976; Quast and Da Silva 1977; Sefah-Dedeh *et al.* 1978; Paulus and Saguy 1980). However, Huang and Bourne (1983) indicate a biphasic softening of vegetables. Since in these studies no indication of correlation with a sensory perception of texture is given, kinetic data obtained can neither be used to optimize sensorial quality (i.e., texture) in thermal processing, nor can the instrumental method replace a sensory evaluation of texture in quality studies.

In the present study the FMC tenderometer and a sensory evaluation are compared for the determination of kinetic parameters of thermal softening of beans (*Phaseolus vulgaris*, subsp. *nanus* Metz., variety *Manteca de Leon*) within the temperature range of 90C to 122C. Despite comments on the tenderometer in literature, the FMC tenderometer was chosen because of its high availability in industry.

MATERIALS AND METHODS

Description of the Products

White beans (*Phaseolus vulgaris*, subsp. *nanus* Metz., variety *Manteca de Leon*) were harvested in the summer of 1991 in Spain. They were stored dry at 15C. Before heat treatment, they were soaked in distilled and demineralized water at 15C for at least 16 h. Preliminary experiments showed that beans reached the maximal moisture content (1.22 g moisture/g dry weight) after 16

h. The mean sizes of the dry (0.19 g moisture/g dry weight) beans were 15.14 mm ± 1.30 mm length, 10.58 mm ± 0.80 mm width and 8.71 mm ± 0.80 mm height. Chemical composition of the beans was 58.46% moisture, 9.44% proteins, 0.55% fat, 3.5% ash and 27.96% carbohydrates.

Heat Treatment

Soaked beans were heated in small cans of 73 mm diameter and 27 mm height, each can containing ± 50 g of beans. The use of flat cans reduced the existence of a temperature gradient in the can during heating so that the difference in heat treatment at the surface and in the center of the cans was minimal. Before sealing cans were filled with distilled and demineralized water; headspace was minimal. Cans were placed in a calibrated oil bath of 30 L (Grant Instruments, Cambridge, Limited HB30). Processing temperatures were chosen to cover the range used in the food industry: 90C, 100C, 110C, 116C and 122C. Samples were heated for predetermined heating times. Immediately after heating, cans were cooled in ice water, to minimize quality destruction during cooling, and stored at 4C for ± 48 h.

Sensory Evaluation

Prior to evaluation, cans that had the same heat treatment were mixed to obtain homogeneous samples. Individual samples (± 20 g of white beans) were served in plastic cups (King disposables, 72 mm diameter and 22 mm height), coded with three random digits. Firmness of white beans was examined under red light to mask differences in appearance. Panel members evaluated firmness by squeezing the beans between their fingers. In order to eliminate distraction and prevent communication among the panelists, evaluations took place in individual booths. The evaluation technique used was a variant of the QDA-method (quantitative descriptive analysis), described by Stone *et al.* (1974). Samples were analyzed using a randomized balanced block design with replication (Cochran and Cox 1957). Twelve trained panel members were asked to evaluate the firmness of at random coded (3 digits) samples by placing a vertical mark on a 14.5 cm scale. Two references were indicated at 1 cm of both ends: the fresh sample, coded 000, and the product processed at 100C during 300 min, coded 300. The distance in mm between the reference coded 000 and the mark placed by the judge was a measure for the intensity of a treatment. During each session, panel members had to analyze six samples of different quality levels processed at the same temperature.

Instrumental Evaluation

Firmness of 200 g processed beans was measured by use of an industrial tenderometer (FMC, model 4011). An FMC tenderometer measures the resistance of the product to compression and shear between two sets of blades. The equipment was calibrated properly. Each heat treatment was evaluated in duplicate with intermediate cleaning in place of the blades. Results were expressed in tenderometer units, i.e., pounds per square inch.

Data-Analysis

Statistical Analysis of the Sensorial Data. First, residues of regression analysis on sensorial data were checked on normal distribution and homoscedasticity. Meeting both requirements, the following statistical techniques could be applied to control whether differences in quality level were detectable: the page-test (O'Mahony 1986), the calculation of R-indices (O'Mahony *et al.* 1980; O'Mahony 1986; Vie *et al.* 1991) and analysis of variance, combined with multiple comparison tests (Wonnacott and Wonnacott 1985). The page-test and the calculation of R-indices were programmed in Turbo Pascal. Anova was performed on the Statistical Analysis System package (SAS 1982). Performance of the panel as a whole was evaluated using factor analysis and by calculating Pearson's correlation matrices. Both methods were programmed using SAS (1982). Results of these statistical techniques have been reported by Van Loey *et al.* (1994).

Reaction Order and Parameter Estimation. The reaction order of the heat induced firmness degradation was determined using nonlinear regression analysis and by examining the tendency of residues. The thermal destruction kinetics was described using the Arrhenius model (Arrhenius 1889) (Eq. 1).

$$k = k_0 \exp(-E_a/RT) \tag{1}$$

The kinetic parameters (E_a, k_{ref}) were estimated by use of three different least squares methods (Ratkowsky 1983; Haralampu *et al.* 1985): two-step linear regression, multiple linear regression and nonlinear regression. All calculations were performed using SAS (1982).

RESULTS AND DISCUSSION

Estimation of the Reaction Order

For each temperature a linear regression analysis was performed with the panel score values on one hand and with the logarithmic values on the other hand as a function of processing time. The tendency of residues as a function of time was a measure to determine if the reaction was zero or first order. The residues of the regression on the sensorial data showed no systematic tendency and higher correlation coefficients were observed for a first order reaction. As a consequence a first order reaction (Eq. 2) was used to calculate kinetic parameters for firmness degradation evaluated by a trained sensory panel:

$$\ln A = \ln A_0 - kt \tag{2}$$

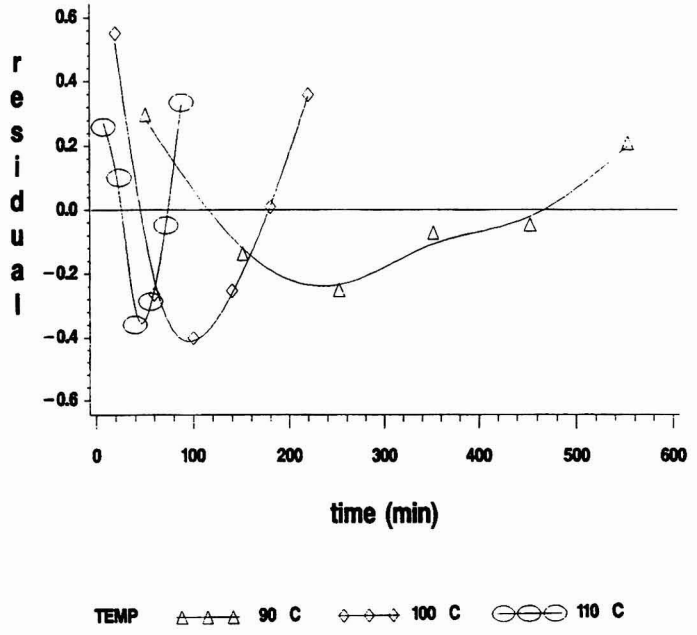

FIG. 1. PLOT OF RESIDUES OF A LINEAR REGRESSION ANALYSIS OF THE LOGARITHMS OF FIRMNESS OF BEANS, EVALUATED BY A TENDEROMETER, AS A FUNCTION OF PROCESSING TIME
Δ = 90C, ◇ = 100C and ○ = 110C.

By assuming a first order reaction for thermal softening of white beans evaluated by the FMC tenderometer, residues showed a systematic tendency (Fig. 1), indicating a misspecification of the model. The rate of thermal softening for beans could not be described by a first order reaction. Based on a study of Huang and Bourne (1983) a biphasic model was used to describe the heat-induced firmness degradation of beans treated for longer heating times. The firmness degradation can be seen as a sum of two independent first order processes. These processes take place simultaneously but with different rate constants.

TABLE 1.

KINETIC PARAMETERS FOR THERMAL SOFTENING OF BEANS EVALUATED BY A SENSORY PANEL AND THE FMC TENDEROMETER

	k_{ref} (10^{-3} min^{-1})	E_a (kJ/mole)
Sensory evaluation	1.65 ± 0.16	130.8 ± 1.3
Tenderometer evaluation		
phase I	20.0 ± 0.60	105.11 ± 20.11
phase II	1.48 ± 0.40	105.18 ± 12.44

Kinetic parameters were obtained by a multilinear regression analysis on the sensorial data and a two-steps linear regression analysis on the FMC tenderometer values.

Determination of Kinetic Parameters

Based on the sensorial firmness evaluation of beans kinetic parameters were estimated using three different least squares methods, two-step linear regression, multiple linear regression and nonlinear regression (Van Loey *et al.* 1994). Large confidence intervals, estimation of unnecessary parameters and regression on regression coefficients made the two-step linear regression inferior to the other two methods. The results of multiple regression were suggested, since calculations were less complex compared to nonlinear regression analysis and indications of accurate estimations were present. By substituting Eq.(1) in (2) and subsequently taking the natural logarithm, formula (3) can easily be derived. Estimated values of k_{ref} and E_a by multiple linear regression are reported in Table 1.

$$\ln\left(\ln\frac{A}{A_0}\right) = \ln k_{ref} - \frac{E_a}{R}\left(\frac{1}{T} - \frac{1}{T_{ref}}\right) - \ln(t) \qquad (3)$$

Equation (3) can be modeled as:

$$Y = a + bX_1 + cX_2$$

where $a = \ln k_{ref}$
$b = -E_a/R$
$c = -1$

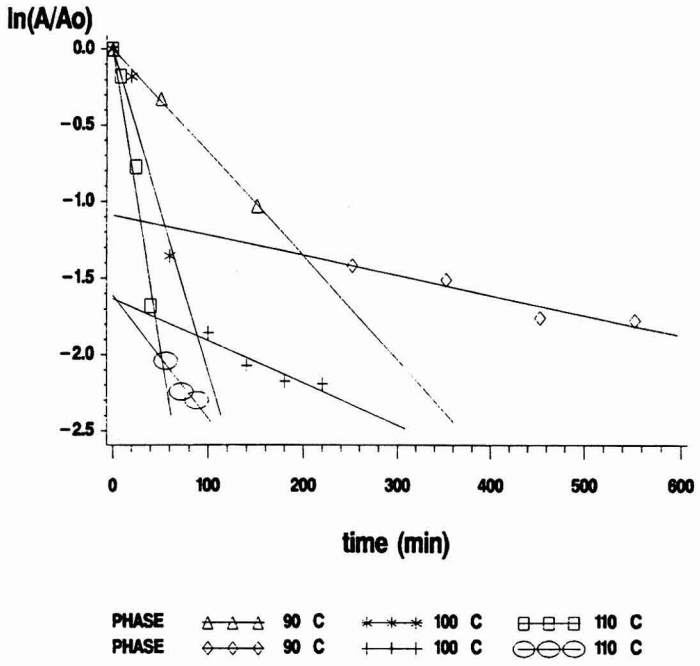

FIG. 2. TWO PHASE APPROXIMATION OF THERMAL SOFTENING OF BEANS
The common logarithms of relative firmness are plotted versus heating times at 90C, 100C and 110C to obtain reaction rate constants at different temperatures.
Phase 1: Δ = 90C, * = 100C, □ = 110C.
Phase 2: ◇ = 90C, + = 100C, ○ = 110°C.

The instrumental evaluation of thermal softening of beans could be described using a biphasic model. For both phases activation energies were estimated using two-step linear regression analysis. First, for each temperature natural logarithms of relative tenderometer values were plotted against heating times to obtain the reaction rate constants k_1 and k_2 of phase 1 and phase 2 of thermal

softening (Fig. 2). For processing temperatures of 116C and 122C the second phase of thermal softening was not observed. These temperatures were eliminated for the two phase approximation. At each temperature, the rate constants of phase 1 and phase 2 are obviously different as can be seen from the different slopes of the regression lines in Fig. 2. The second step in the Arrhenius model is to regress the natural logarithm of the rate constants, $\ln k_1$ and $\ln k_2$, versus the reciprocal of the absolute temperature, 1/T, to obtain the estimates of the activation energies from the slope index of the regression lines (cfr. Eq. 1)(Table 1). Regression lines are more or less parallel so that the activation energies for both phases of firmness degradation of beans measured by a tenderometer are equal (105 kJ/mole). This contradicts the results of Huang and Bourne (1983). They indicated higher activation energies for the first phase than for the second (E_{aI} = 104.1 kJ/mole and E_{aII} = 53.9 kJ/mole). Huang and Bourne (1983) used a back extrusion cell, 10.2 cm i.d. by 12 cm height with a 4 mm annulus, mounted in the Instron Universal testing machine to evaluate heat induced firmness degradation of vegetables.

Literature data on the kinetics of hardness degradation are given in Table 2. Differences in variety and measuring instruments do not permit a strict comparison of the data. What type of force and how, direction and rate, it is applied is very critical. The FMC tenderometer and the Kramer shear press, used by Quast and Da Silva (1977), are both multiple blade shearing devices. The FMC tenderometer consists of a grid of 19 blades to compress and then shear and extrude a sample through a second grid of 18 blades. The reaction grid is also allowed to rotate, but the rotation is resisted by a pendulum attached to this grid with a weight on the end. The force exerted by the sample during compression, shearing and extrusion is shown by the angular movement of the pendulum. Because of the rotational shearing action the sample is not compressed uniformly along the length of the blades (Voisey and Nonnecke 1971; Moskowitz 1987). Quast and Da Silva (1977) measured the degree of cooking on a Kramer shear press with a CS-1 shear cell. The Kramer shear press uses a stationary rectangular box with slots in the bottom to hold the sample and a moving probe composed of ten bars to drive through the test specimen (Kramer and Szczesniak 1973). An important difference between both measuring instruments is that in the Kramer shear press linear instead of rotary motion of the blades is used.

Comparison Sensory and Tenderometer Values

Performance of the individual judges (validity and reliability) was evaluated using two-out-of-five tests, by presenting duplicate test samples and replications. None of the panel members showed serious deviations in their judgments during

TABLE 2.

REVIEW ON KINETIC DATA FOR THERMAL SOFTENING OF PEAS AND BEANS

product	E_a(kJ/mole)	Method	Reference
white beans (phase II)	53.92	Instron Universal testing machine	Huang and Bourne 1983
peas (phase II)	91.96-100.32	Instron Universal testing machine	Huang and Bourne 1983
	176.4	Kramer shear press	Quast and Da Silva 1977
black beans	148.39	Kramer shear press	Quast and Da Silva 1977
brown beans	156.75	Kramer shear press	Quast and Da Silva 1977
soya beans	181.83	Kramer shear press	Quast and Da Silva 1977

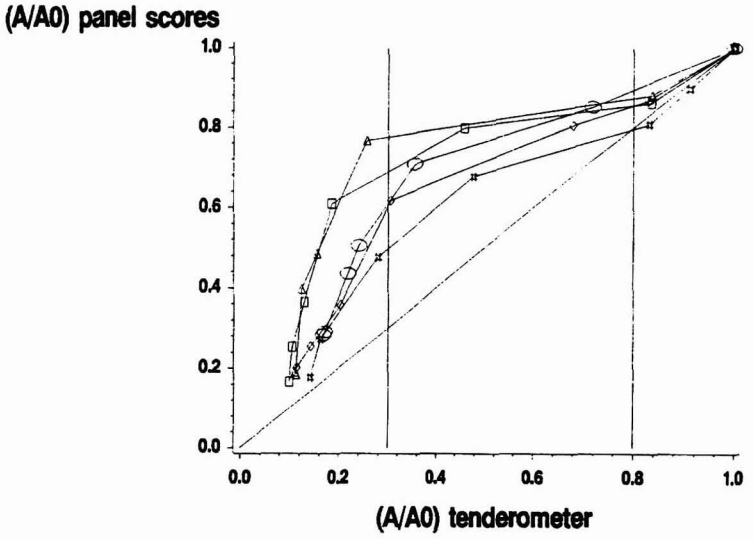

FIG. 3. PLOT OF RELATIVE PANEL SCORES VERSUS RELATIVE TENDEROMETER VALUES FOR FIRMNESS EVALUATION OF BEANS TREATED AT DIFFERENT TEMPERATURES
○ = 90C, Δ = 100C, □ = 110C, ◇ = 116C and # = 122C.

the sessions. Factor analysis and the calculation of Pearson's correlation matrices examined the performance of the panel as a whole. The high correlation of the vectors representing different panel members with factor 1 and the small angles between the vectors prove that all panellists judged thermal softening in the same way.

The correlation coefficient for a tenderometer evaluation versus a sensory evaluation of thermal softening of beans was 0.88. However, the limitations of this statistical parameter must be considered (Szczesniak 1968; Kramer 1969). The correlation coefficient is highly dependent upon the range of values covered, the number of samples involved and the magnitude of variations within the block of samples. Therefore it would be desirable if authors reporting correlations between objective and sensory texture measurements would give scatter diagrams from which the range of values covered and the number of samples tested could easily be seen (Szczesniak 1968) (Fig. 3). In literature correlation coefficients for a pea tenderometer measurement versus a sensory assessment are within the range of 0.62 to 0.97 (Voisey and Nonnecke 1973). Factor analysis was applied to examine the correlation between sensory evaluation and tenderometer firmness measurement of thermally treated beans. The length of the vectors in the biplot presentation indicates a very good reconstruction in the two dimensional plane.

The vector representing the tenderometer evaluation is highly correlated with those representing the panel members.

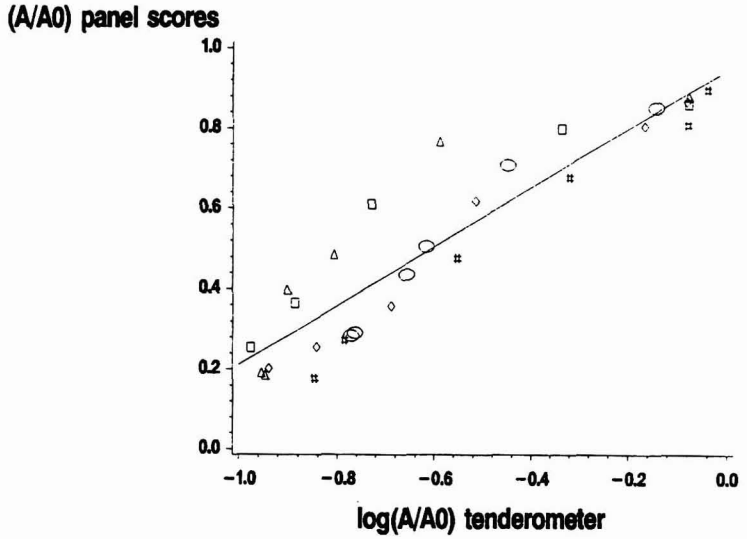

FIG. 4. RELATION BETWEEN MEAN SENSORY ESTIMATES AND TENDEROMETER VALUES OF FIRMNESS EVALUATION OF BEANS TREATED AT DIFFERENT TEMPERATURES

o = 90C, Δ = 100C, □ = 110C, ◇ = 116C and # = 122C.

The straight line represents the least squares fit of the loglinear function.

However when tenderometer data are plotted versus sensory data (Fig. 3) a difference in firmness evaluation according to the measuring technique can be noticed. Figure 3 can be divided in three parts. For short processing times a tenderometer evaluation and a sensory evaluation give analogous results. In the second part the tenderometer scores decrease faster than those of the sensory panel, which indicates a higher sensitivity of a tenderometer firmness evaluation. For longer processing times the sensory panel seems to be more sensitive to a further firmness decay. G.T. Fechner (Kramer and Szczesniak 1973) suggested a logarithmic function S=k.log(I) that could be used to relate the sensory magnitude S to the physical intensity I. This relationship (Fig. 4) can explain lower sensory discrimination power at higher intensities. Coefficients of determination (R^2) of a linear regression analysis performed at each temperature separately were 0.94 at 90C, 0.81 at 100C, 0.90 at 110C, 0.98 at 116C and 0.99 at 122C, and for all data points 0.87. A shift in temperature can be noted from Fig. 4. For samples thermally treated at 100C and 110C panel scores are

underestimated using Fechner's law, whereas for higher temperatures panel scores are overestimated.

CONCLUSION

A first order reaction was assumed for thermal softening of beans measured by a sensory panel while a tenderometer firmness evaluation was described using a biphasic model. Kinetic parameters were estimated using a multilinear regression analysis on the sensorial data whereas a successive two-step linear regression analysis estimated activation energies of both phases of the two phase approximation. Because of the difference in kinetic parameter estimates, the FMC tenderometer could not be used to determine kinetic parameters of thermal softening of white beans to be used for sensory process optimization. Although a high correlation coefficient was found using Fechner's law, further research is needed to examine the observed shift in temperature.

NOMENCLATURE

a, b, c	Parameters to be estimated
A	Quality parameter
E_a	Activation energy (J/mole)
k	Rate constant
n	Reaction order
R	Universal gas constant = 8.3065 J/(K mole)
T	Temperature (C or K)
t	Time (min)

Subscripts

0	Initial value
ref	Reference

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INFLUENCE OF 7S AND 11S GLOBULINS ON THE EXTRUSION PERFORMANCE OF SOY PROTEIN CONCENTRATES

L. NING[1] and R. VILLOTA[1]

Department of Food Science
University of Illinois
1304 W. Pennsylvania
Urbana, IL 61801

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ABSTRACT

The 7S and 11S fractions of soy protein were isolated from soy flour and recombined with soy protein concentrate at various levels to modify their ratio in different formulations. The role of each fraction on the extrusion performance and texturization behavior of soy proteins was evaluated using twin-screw extrusion. Both 11S and 7S fractions were found to have significant influence on the degree of texturization of soy protein. In particular, the 11S protein appeared to favor expansion and water holding capacity of the finished product, while an 11S/7S ratio of 1.5 in the feed formulation resulted in a product with the best textural characteristics under the selected extrusion conditions investigated.

INTRODUCTION

Extrusion has been used as a technology to texturize defatted soy protein, obtaining tissue-like structures. Texturization of vegetable proteins has been extensively used to create meat analogues and extenders, greatly improving the utilization of soy proteins. Although extrusion texturization of soy protein has been subjected to a wide investigation, much remains unclear with regard to the molecular mechanisms involved in structure formation. Some common molecular structure requirements have been recommended for proper protein texturization including high molecular weight, long linear chain length, high

[1]Send correspondence to Dr. Ricardo Villota, Kraft General Foods, 801 Waukegan Rd., Glenview, IL 60025; or Dr. Luping Ning, Univ. of Illinois, Dept. Food Science, 382 A.E.S.B., 1304 W. Pennsylvania, Urbana, IL 61801.

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degree of linear symmetry, absence of a high ratio of bulk side chains and a high degree of polarity (Consolacion and Jelen 1986). Hagan *et al.* (1986) combined enzymatically hydrolyzed soy protein with unhydrolyzed soy protein to determine the effects of protein molecular weight distribution on its performance during texturization by twin-screw extrusion. Their results suggested that high protein molecular weight is essential for adequate product texturization, while small molecular weight peptides are not extensively involved in texture formation.

There is much controversy over the types of chemical bonds involved in the texture formation of soy protein. Disulfide bonding together with noncovalent interactions have been shown to be the major chemical forces involved in soy spun fiber integrity (Chiang and Sternberg 1974; Kelley and Pressey 1966). Burgess and Stanley (1976) postulated that the chemical forces involved in texturized soy protein were between 54 Kcal/mol and 83 Kcal/mol, and that disulfide bonds were negligible in texture formation, since they were disrupted during extrusion. Thus, they suggested intermolecular peptide bonds as being the primary force responsible for the stabilization of texturized soy protein. This hypothesis was supported by the work done by Simonsky and Stanley (1982), in which free amino groups were found to be important for protein texturization. However, Sheard *et al.* (1986) refuted the idea proposed initially by Burgess and Stanley (1976) by concluding that disulfide bonds and hydrophobic interactions are the major chemical forces involved in stabilizing the extrudates. Jeunink and Cheftel (1979), on the other hand, claimed that disulfide bonds and/or noncovalent interactions played a role in protein texturization only when they were properly positioned. It seems that more work needs to be done in order to fully understand the formation of texturized protein products.

Endogenous protein fractions of soy are known to provide the major skeleton structure of texturized products. Stanley (1989) described the influence of extrusion on soy proteins as a process that disassembles and converts them into a physically homogeneous matrix, and then recombines and reconnects them chemically into a fibrous, oriented structure with unique characteristics. Rhee *et al.* (1981) reported that the shear values of extrusion-texturized soy were significantly increased at high protein concentrations. Cumming *et al.* (1973) found that insolubilization of the water-soluble proteins and dissociation of the remaining proteins into subunits occurred after soy extrusion. 7S and 11S globulins represent the two major fractions of soy proteins, constituting about 70% of the total water-soluble proteins in soy. The physicochemical characteristics of 7S and 11S globulins have been well documented (Wolf 1970). 7S and 11S globulins exhibit a quaternary structure of high molecular weight subunits, being stabilized by hydrophobic interactions among the nonpolar residues. They can undergo a complex pattern of association-dissociation while being heated. German *et al.* (1982) reported on the formation of a soluble complex between

7S and 11S globulins during thermal processing, which significantly modified the heat stability and solubility of whole soy protein. Although it has been assumed that the texture formation of fabricated soy protein is a result of alterations in the protein structure, little is known about the role of 7S and 11S globulins on the extrusion performance of this protein. In this study, we attempted to characterize the role of each soy protein fraction on the texturization behavior and extrusion performance of soy protein.

MATERIALS AND METHODS

Fractionation and Isolation of 7S and 11S Fractions from Soy Flour

Soy 7S and 11S globulin-enriched fractions were prepared from soy flour (Soyafluff 200W, Central Soya Co., Fort Wayne, IN) according to the method described by Howard *et al.* (1983). This method involves the isolation of soy protein from soy flour through aqueous extraction, followed by selective pH adjustment and centrifugation. The extraction medium contained 0.03M NaCl and 0.77mM $NaHSO_3$. The 7S and 11S purities were determined on the basis of their sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis profiles by following the method of Howard *et al.* (1983). Gel electrophoresis was performed as described by Laemmli (1970). Quantitation of the individual species was obtained by densitometric scanning of the SDS gel profiles. The total 7S globulin is a sum of α, α' and β subunits and the total 11S globulin is a sum of acidic and basic subunits. The purities of the 7S and 11S globulin fractions were 80% and 90%, respectively.

Twin-Screw Extrusion of Soy Protein Concentrate with Various 11S/7S Ratios

The isolated 7S and 11S soy protein fractions were recombined with commercial soy protein concentrate (Promax, Central Soya Co., Fort Wayne, IN) to formulate new feed materials. The new feeds, containing various 11S/7S ratios ranging from 0.51 to 2.35, were extruded using a Werner & Pfleiderer twin-screw extruder Model ZSK-30 (Werner & Pfleiderer Corp., Ramsey, NJ). The barrel temperatures of the five zones from the feed section to the die exit were set at 60C, 100C, 140C, 150C and 150C, respectively. Extrusion was carried out at 20% moisture content with a dry feed rate of 142 g/min. The screw speed was 350 rpm. A dual orifice die (8 mm diameter) was used for the experiment. The extrudate was dried in an air drier at room temperature for 8

h, a portion of which was then ground and sifted through different size sieves. Measurements in terms of physical properties, rheological properties, and solubility in selected solvents were conducted on the extrudates.

Expansion Ratio (ER)

Expansion ratio, the ratio of the diameter of the extrudate divided by the diameter of the die orifice, was monitored after drying and cooling of the extrudate, and reported as an average of 20 measurements.

Bulk Density (BD)

Bulk density of whole extrudates was determined by placing 10 g of sample in a 250 ml graduated cylinder. The cylinder was then filled with sand and the volume of sand was measured. The volume of the extrudate was determined by subtracting the sand volume from 250 ml. The bulk density was expressed as grams of extrudate per ml.

Water Holding Capacity (WHC)

WHC of whole extrudate was measured by weighing approximately 10 g of extrudates and allowing them to hydrate at 25C for 2 h. The rehydrated extrudates were then drained and weighed. WHC was expressed as ml of water retained per gram of sample.

Nitrogen Solubility Index (NSI)

NSI measurements were carried out according to the A.A.C.C. methods (AACC 1975).

Nitrogen Solubility in Various Solvents

Protein solubilities were determined in the following solvents: 0.5 M NaCl (agent disrupting electrostatic interactions), 2% sodium dodecyl sulfate (SDS, agent disrupting noncovalent interactions), and 0.5 M 2-mercaptoethanol (ME, agent disrupting disulfide bonds). To 100 ml of a given solvent were added 2.0 g of ground extrudate. Samples were extracted for 4 h at room temperature

under moderate agitation, followed by centrifugation at 1000 × g for 15 min and subsequent filtration through Beckman 40 filter paper. Filtrates were analyzed for protein content by The Kjeldahl method (AACC 1975).

Scanning Electron Microscopy (SEM)

Extrudate samples were mounted on a pin stub, coated with gold-palladium and examined on a lower stage of an ISI-DS-130 scanning electron microscope with an accelerating voltage of 10 KV.

Differential Scanning Calorimetry (DSC)

A Perkin-Elmer DSC-4 differential scanning calorimeter was used to investigate the effect of the 11S/7S ratio on the temperature of denaturation of the 7S and 11S globulins. A heating rate of 10C/min was applied to 15–20 mg of a 20% protein solution in hermetically sealed aluminum pans. Indium metal was used for temperature calibration.

Heat-Induced Gels

Heat-induced gels of both feed materials and isolated soy proteins having a different 11S/7S ratio were prepared according the method of Ning *et al.* (1990).

Texture Analysis

Rheological characteristics of rehydrated extrudates and heat-induced soy protein gels were monitored using an Instron (Instron Universal Testing Machine, Model 1122). The samples were weighed prior to rehydration. A 4.0 cm rehydrated extrudate was compressed twice with a flat head compression plunger (5.5 cm in diameter) to 70% of its original diameter.

The cross-head speed and chart speed were set at 50 mm/min and 500 mm/min, respectively. Textural parameters in terms of elasticity, cohesiveness, hardness, gumminess and chewiness were measured according to the method described (Breene 1975).

RESULTS AND DISCUSSION

Physical characteristics of extrudates as listed in Table 1 showed an increase in expansion ratio with an increase in the 11S/7S ratio in the feed formulation. Bulk density, as expected, negatively correlated with expansion ratio (BD vs. ER, $r = -0.975$). SEM micrographs showed that extrudates containing high levels of the 7S protein fraction had a dense and compact structure, while the 11S enriched extrudates had a spongy texture with enlarged air cells. Adequate protein fiber alignment was observed for products having an 11S/7S ratio of 1.0 and 1.5 (Fig. 1). The increased water holding capacity of the feed with high 11S concentration may be responsible for these changes (Table 1). Similar results were reported by Yao *et al.* (1988), who found that the water-imbibing capacity, the consistency coefficient and the rheological properties of soy protein isolate were greatly improved as the 11S concentration increased from 35% to 100%. Since product expansion is partially a result of the flash vaporization of water, the amount of water that is physically held within the soy protein matrix and its state of existence are expected to be major factors controlling product expansion. The elasticity of the protein dough and its ability to maintain a deformation are also critical to structure formation. The increased WHC of the feed material having a high 11S concentration apparently favors the entrapment of water during extrusion.

TABLE 1.
PHYSICAL AND RHEOLOGICAL PROPERTIES OF EXTRUDATES HAVING A DIFFERENT 11S/7S RATIO

11S/7S	E.R.	B.D. (g/ml)	WHC(Feed) (g water/g)	WHC(Extrudate) (g water/g)	Cohesiveness	Elasticity
0.51	1.19	1.05	0.84	0.82	0.50	0.69
0.74	1.25	0.98	1.11	1.28	0.57	0.78
1.06	1.38	0.86	1.44	1.62	0.64	0.81
1.26	1.42	0.90	1.48	1.64	0.66	0.83
1.51	1.37	0.78	1.63	1.67	0.69	0.86
1.96	1.72	0.51	1.78	1.76	0.65	0.85
2.35	1.88	0.43	1.92	2.12	0.73	0.88

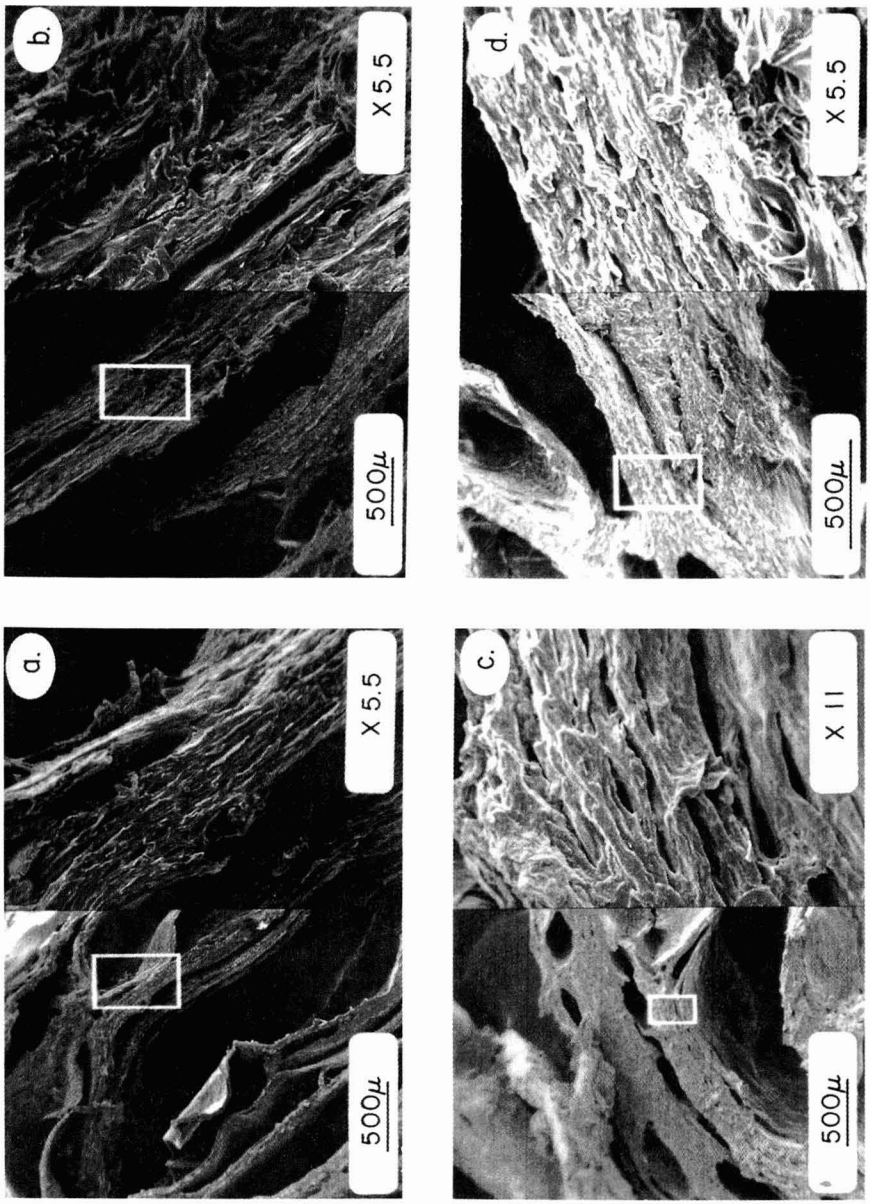

FIG. 1. SCANNING ELECTRON MICROGRAPHS OF EXTRUDATES PREPARED WITH DIFFERENT 11S/7S RATIOS

(a) 11S/7S = 1.0, (b) 11S/7S = 1.5, (c) 11S/7S = 2.0, (d) 11S/7S = 2.35

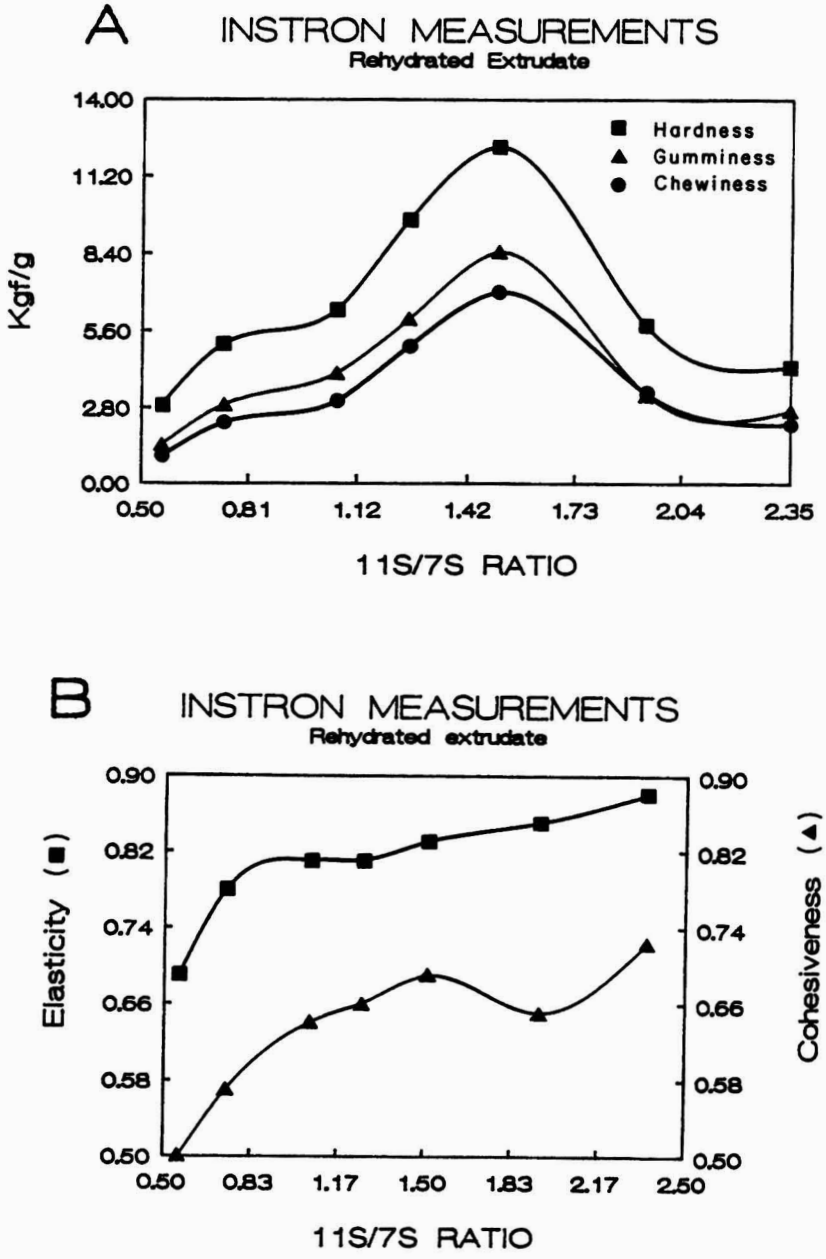

FIG. 2. EFFECT OF 11S/7S RATIO ON TEXTURAL ATTRIBUTES OF REHYDRATED EXTRUDATES
(A) hardness, gumminess, chewiness; (B) elasticity, cohesiveness.

The water holding capacity of the whole extrudates (Table 1), and the elasticity and cohesiveness of the rehydrated extrudates (Fig. 2B) were positively correlated (WHC vs. Cohesiveness, r = 0.970; WHC vs. Elasticity, r = 0.979); they all increased with an increase in the 11S/7S ratio in the feed formulation. It is suggested that the water holding capacity of the whole extrudate is directly related to its internal porosity due to the capillary action of an aligned fibrous protein network. As previously discussed, the dense and compact structure as obtained in 7S-enriched extrudates may retard water penetration, resulting in poor water hydration of the product. On the other hand, the relative spongy and porous texture as observed in 11S-fortified extrudates will favor the adsorption of water. Elasticity and cohesiveness are rheological parameters measuring the ability of the rehydrated extrudate to resume the original shape after being compressed. They are controlled by the internal microstructure of the extrudate and/or their ability to swell under the action of solvent water. As expected, the porous internal structure of the 11S-enriched extrudates with regular and thin cell walls allowed water to pass through easily, resulting in increased elasticity and cohesiveness.

Rheological parameters of rehydrated extrudates as measured by Instron showed similar trends. The hardness, gumminess and chewiness all increased with an increase in 11S concentration up to a maximum ratio of approximately 1.5 (Fig. 2A). A positive correlation was found between the rheological properties of rehydrated extrudates (Fig. 2A) and protein solubility in chemical reagents, including sodium chloride (NaCl), sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (ME)(Chewiness vs. NSI_{SDS}, r = 0.814; Chewiness vs. NSI_{ME}, r = 0.746; Chewiness vs. NSI_{NaCl}, r = 0.753)(Fig. 3). Protein solubility in chemical reagents revealed the nature and extent of protein interactions in the extrudate; solubility in sodium chloride, sodium dodecyl sulfate and 2-mercapto-ethanol correspond to the extent of electrostatic interactions, noncovalent interactions and disulfide bonds, respectively. Results suggest that all these molecular forces are involved in stabilizing and strengthening the structure of extrusion-texturized soy protein; however, noncovalent interactions appeared to play the most important role.

As compared with the rheological parameters (Fig. 2A), an opposite trend was found with regard to the effect of 11S/7S ratio on nitrogen solubility index (NSI) of both ground and whole extrudates (Chewiness vs. NSI_{Ground}, r = −0.879; Chewiness vs. NSI_{Whole}, r = −0.787). In each case, the lowest protein solubility was observed for samples having an 11S/7S ratio of 1.5 (Fig. 4). Since during thermal extrusion soy proteins are subjected to high temperature, pressure and shear in a low moisture environment, the physical state of these complex and reactive protein molecules will undergo drastic changes. Heat denaturation followed by aggregation are expected to occur during this process. The aggregation process, which involves interactions among the unfolded

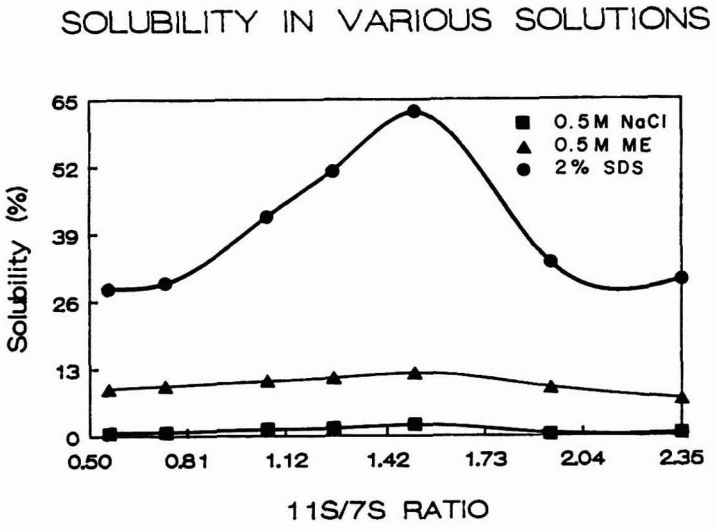

FIG. 3. NITROGEN SOLUBILITY OF EXTRUDATES IN VARIOUS SOLVENTS AS AFFECTED BY 11S/7S RATIO

polypeptide chains via various chemical forces is necessary for the proper formation of a continuous protein network. Therefore, factors affecting the denaturation and aggregation processes may have a strong influence on the extrusion behavior of soy protein. After studying the thermal denaturation and aggregation behavior of soy protein, Hermansson (1978) found that the 11S fraction can easily precipitate out of solution and is alone responsible for the reversible aggregation of soy protein. A more detailed explanation on this subject was provided by Damodaran and Kinsella (1982), who stated that the thermal dissociation of the 11S globulins produces acidic and basic subunits; however, only the dissociated basic subunits can form soluble aggregates among themselves and then associate in the form of insoluble aggregates primarily via hydrophobic interactions. In our study, DSC results showed that the denaturation temperature of both 7S and 11S decreased with an increase in 11S concentration (Fig. 5). This indicates that 7S and 11S globulins denatured more easily at high 11S levels, which facilitates the unfolding and associated interactions among unfolded polypeptide chains. On the other hand, Hermansson (1978) pointed out that the 7S fraction may aggregate upon heating before unfolding without prior conversion into subunits, or alternatively, it may form unstable dissociation products that aggregate very rapidly relative to the dissociation and unfolding steps. Moreover, this phenomenon may become more important in the extrusion process where drastic levels of heat and mechanical energy are applied. Thus,

the lack of interactions among polypeptide chains of the 7S-enriched extrudates may limit the formation of a protein network, hence resulting in poor rheological properties.

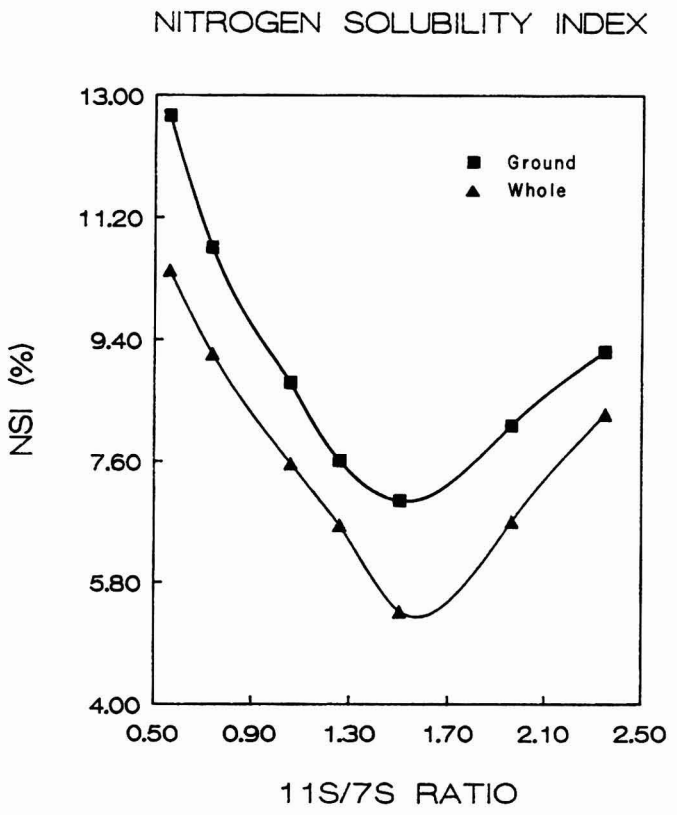

FIG. 4. NITROGEN SOLUBILITY INDEX OF EXTRUDATES AS AFFECTED BY 11S/7S RATIO

The maximum rheological parameters as observed for samples having an 11S/7S ratio of 1.5 (Fig. 2A) may be closely related to the unique interactions between the dissociated 7S and 11S subunits. The mechanisms behind 7S and 11S globulins interactions have been discussed by German *et al.* (1982). While soy protein is under heat treatment, 11S and 7S globulins dissociate into subunits. Hence, the positively charged basic subunits of glycinin (11S) will interact with negatively charged acidic subunits of conglycinin (7S) via electrostatic interactions and/or hydrophobic interactions, leading to the

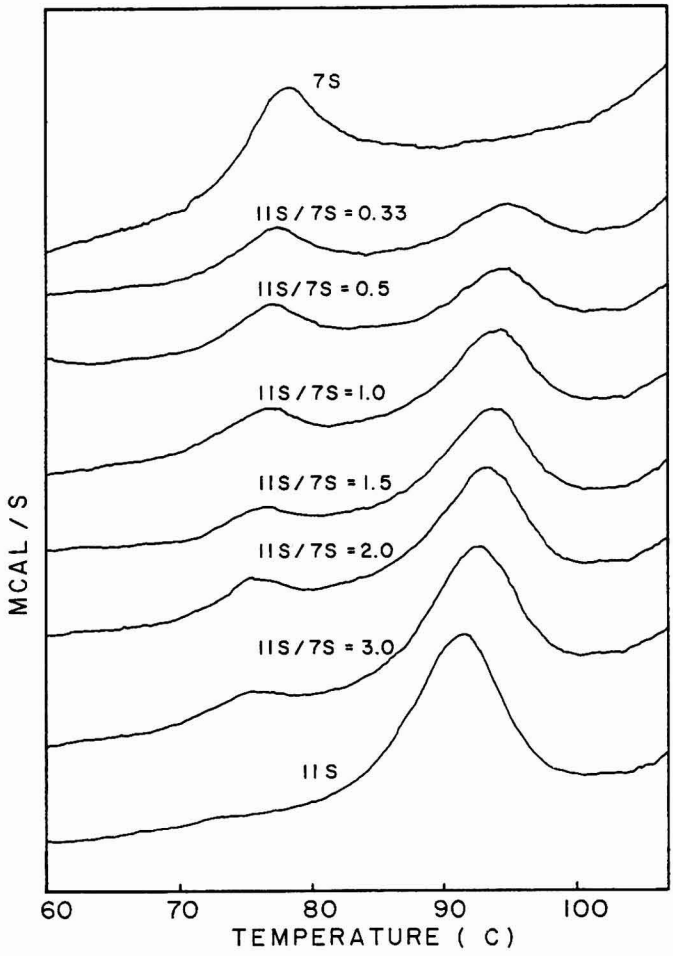

FIG. 5. DENATURATION TEMPERATURES OF SOY PROTEINS WITH VARIOUS 11S/7S RATIOS AS DETERMINED BY DSC

formation of a soluble complex. The soluble aggregate becomes stabilized due to the existence of a net charge on their surface, acting to repel each other. However, as the 11S/7S ratio changes, the complex may possess a zero net charge at the ionic conditions encountered. Hence, the basic subunits of 11S globulin may precipitate along with conglycinin via molecular interactions, forming large insoluble aggregates, which may serve as the skeleton of the protein network. The nitrogen solubility index (NSI) of ground extrudates is known to be directly associated with the degree of protein denaturation

(Cumming *et al.* 1973; Harper 1981). Lee and Rha (1979) stated that the development of numerous protein-protein interactions or the formation of dense aggregates caused protein molecules to precipitate with loss of water solubility. Thus, nitrogen solubility index (NSI) is also an indication of the degree of protein-protein interactions within the extrudate, whereby a low NSI may indicate a high degree of protein-protein interactions. The lowest NSI, occurring at an 11S/7S ratio of 1.5, may be another indication of the highest degree of insoluble aggregates formed under these conditions (Fig. 4). The corresponding maximum amount of noncovalent interactions, disulfide linkages and electrostatic interactions determined in samples having an 11S/7S ratio of 1.5 will explain the best rheological properties of the rehydrated extrudates (Fig. 2A).

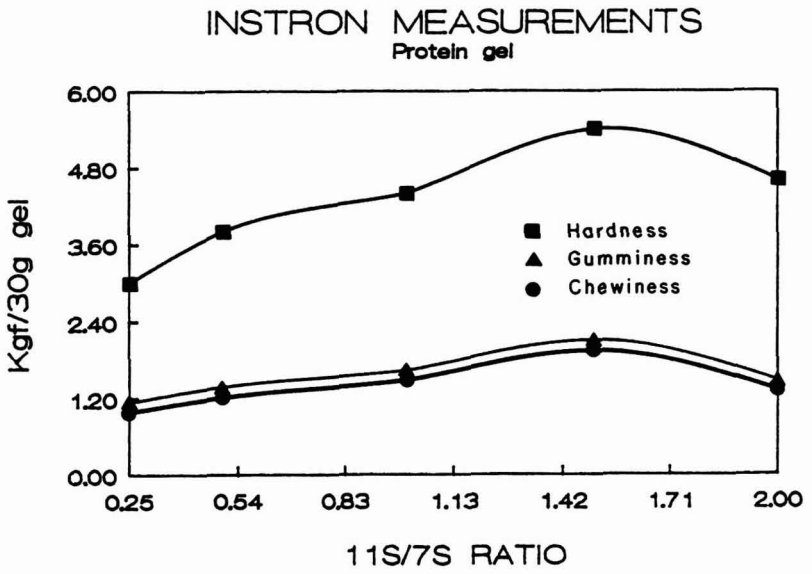

FIG. 6. TEXTURAL MEASUREMENTS OF PROTEIN GELS AS AFFECTED BY 11S/7S RATIO

By studying the gelation behavior of soy protein, the molecular mechanisms and chemical forces involved in protein network formation can also be explored. Utsumi and Kinsella (1985) studied the molecular forces involved in heat-induced gels made from soy 7S, 11S and soy protein isolate. Their results indicated that electrostatic interactions and disulfide bonds were involved in the formation of 11S globulin gels, mostly hydrogen bonding in the 7S globulin gel

and hydrophobic interactions in the soy protein isolate gel. Saio *et al.* (1969) found that the 11S globulin formed a harder and more elastic gel than the 7S fraction. However, it is not possible to obtain the proper gel structure with only one of these two major globulins. Babajimopoulos *et al.* (1983) demonstrated that the gel viscosity of soy protein isolate was significantly higher than that of the combined 7S and 11S fractions, which leads to the conclusion that the complex formed between subunits of 7S and 11S globulins upon heating results in a better and stronger protein network. In our study, the optimum rheological parameters of heat-induced gels occurred in samples having an 11S/7S ratio of 1.5 (Fig. 6), which correlated well with the rheological properties of the rehydrated extrudates (Fig. 2a). This reaffirms that interactions between 11S and 7S affected the texturization process of soy protein.

CONCLUSIONS

Significant differences in the texturization behavior of soy protein during extrusion were achieved by adjusting the 11S/7S ratio in the feed formulation. Both 7S and 11S fractions were found to have a certain degree of influence on the texture formation of soy protein. The 11S protein appeared to favor the expansion and water holding capacity of the finished product. A negative effect on soy protein texturization was observed when the 7S protein fraction was incorporated at high levels. The 7S and 11S globulins interacted with each other during extrusion processing, with the best textural characteristics of the finished product being obtained with a feed formulation having an 11S/7S ratio of 1.5. Among all the molecular forces responsible for texture formation investigated, noncovalent interactions, such as hydrophobic interactions and hydrogen bonding appeared to be dominant. Disulfide bonds were also found to make a contribution to texture formation.

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BOOK REVIEWS

THE LACTIC ACID BACTERIA. Edited by Eng-Leong Foo, Hugh G. Griffin, Roland Möllby, and Carl-Göran Hedén, Horizon Scientific Press, 15 Barnham Broom Read, Wymondham, Norfolk NR18 ODF, England. 1993. 120 pages.

Each week during May and June of 1993 at least two papers on lactic acid bacteria were distributed electronically to more than 150 people in 22 countries. Participants, after examining a paper, had the opportunity to "discuss" it via computer. These two phases constituted *The First Lactic Acid Bacteria Computer Conference*, which was sponsored by the Biofocus Foundation of Stockholm, Sweden and organized by the Lactic Acid Bacteria Electronic Network, Unesco Microbial Resources Center, and the Department of Bacteriology at the Karolinska Institute; all three groups are in Stockholm. The 18 conference papers and associated discussions (some papers did not generate discussions) are the contents of this book.

There is no central theme (other than lactic acid bacteria) to the contents of the book; it is not evident how contributions and contributors were selected. Subjects of the contributions include meat fermentations, dairy fermentations, proteases and peptidases of lactic acid bacteria, nisin, a fermented milk product for use by constipated geriatric patients, bacteriophage problems, lactose operon expression, inhibition of pathogens, lactic acid production, the sugar cane industry, the malolactic fermentation, immobilized lactic acid bacteria, European culture collections, and cell-wall polymers. Most papers are mini-reviews, several present original research results, and only a few exceed five printed pages including references and discussion. Based on the address of the senior author, one paper came from Belgium, two from Brazil, one from Cuba, two from France, one from The Netherlands, three from Norway, one from Sweden, one from Spain, two from Turkey, one from the United Kingdom, and two from the United States.

Although this book was generated in a unique manner, as might be expected, the individual papers vary considerably in quality of content and of written presentation. It is unfortunate that the editors did not further improve several of the presentations by authors not fluent in English. Furthermore, use of nomenclature for the lactic acid bacteria is not uniform throughout the book. Also, the two-page index is quite inadequate.

Presentations on proteases and peptidases of the lactic acid bacteria are among the best in the book. The papers on a fermented milk product used to treat constipation in geriatric patients and on immobilized lactic acid bacteria are unique and interesting contributions.

Persons doing research on lactic acid bacteria are probably familiar with most of what is in the book. If they are not, this book is not the source for an in-depth discussion of the various areas of research or application related to these bacteria. Consequently, the book probably would be of greatest interest to microbiologists and food scientists working in other areas who want to easily become familiar with some current developments regarding the lactic acid bacteria.

ELMER H. MARTH, PhD, RS

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FNP PUBLICATIONS IN FOOD SCIENCE AND NUTRITION

Journals

JOURNAL OF FOOD LIPIDS, F. Shahidi
JOURNAL OF RAPID METHODS AND AUTOMATION IN MICROBIOLOGY, D.Y.C. Fung and M.C. Goldschmidt
JOURNAL OF MUSCLE FOODS, N.G. Marriott and G.J. Flick, Jr.
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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 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:

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

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RIZVI, S.S.H. 1986. Thermodynamic properties of foods in dehydration. In *Engineering Properties of Foods*, (M.A. Rao and S.S.H. Rizvi, eds.) pp. 133–214, Marcel Dekker, New York.

MICHAELS, S.L. 1989. Crossflow microfilters ins and outs. Chem. Eng. *96*, 84–91.

LABUZA, T.P. 1982. *Shelf-Life Dating of Foods,* pp. 1–9, Food & Nutrition Press, Trumbull, CT.

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