

# Mechanical and Structural Evaluation of Texturized Soy Proteins of Varying Protein Content

M. KAZEMZADEH,<sup>1</sup> K. C. DIEHL, JR.,<sup>2</sup> K. C. RHEE,<sup>3</sup> and P. F. DAHM<sup>4</sup>

## ABSTRACT

Cereal Chem. 63(4):304-310

Defatted soy flour and mixtures of soy protein isolates and insoluble peanut carbohydrates varying from 20 to 80% isolate were extruded in a Wenger X-5 extruder. Experiments were performed on the extrudates using a Food Technology Corp. CS-1 cell, an Ottawa texture measurement system 10-cm<sup>2</sup> wire cell, and the USDA Category I Tester (CIT) test. The samples were also examined by scanning electron microscopy and transmission light microscopy. Parameters from the tests on the isolate-carbohydrate extrudates were correlated with percent soy protein isolate.

When the defatted soy flour extrudates were compared to the isolate-carbohydrate mixtures mechanically and microscopically, the soy extrudates, which had a protein content of 57.6%, were generally most closely related to the soy protein isolate-insoluble carbohydrate mixtures, which contained an isolate content in the 50-60% range. All of the instrumental parameters were highly correlated to the soy protein isolate content. A pronounced increase in all of the measured parameters occurred between 40 and 70% soy protein isolate content.

Instrumental measurement of texturized plant proteins (TPP) has been used frequently to quantitatively characterize the texture of TPP or to assess the effects of processing variables or additives on TPP texture. A variety of devices, usually mounted on a universal testing machine, have been used to determine parameters that have then been defined by the investigators as the instrumental texture measurements. Shear or extrusion cells have usually been used, although geometries that yield more basic rheological properties have also been used.

Using measurements obtained with the Warner-Bratzler and the Food Technology Corp. CS-1 cells, Cumming et al (1972) and Maurice and Stanley (1978) found high correlations between the measured values and the processing temperature used during extrusion of defatted soy flour. In the latter study, the instrumental texture measurements were also highly correlated to sensory parameters.

Breene and Barker (1975) used values determined with a 10-cm<sup>2</sup> Ottawa texture measurement system (OTMS) extrusion cell to compare ground beef, textured soy products, and a mixture of ground beef and textured soy. These measurements were used to separate the materials into textural classes. Using the same methodology, Loh and Breene (1977) later related the instrumental test results to sensory evaluation data.

Taranto et al (1978a,b) used a Wenger X-5 extruder to extrude defatted soy flour at various levels of moisture content, screw speed (rpm), die size, temperature at the die, back pressure, and feed rate. The extrudates were tested using an OTMS extrusion cell, and the results (defined as stress and resilience) were used to predict which processing conditions would yield desired textures. The texture measurements were corroborated by microscopic studies. Similarly, Kazemzadeh (1980) used the same measurements to examine the effect of varying pH and last-stage barrel temperature on extrudate texture. The extrudates were also examined using scanning electron microscopy. Microscopic examinations such as these have been useful in visualizing changes in extrudate microstructure caused by chemical and process variables.

Because several instrumental texture devices have been used to measure TPP textural properties, one of the objectives of this study was to compare at least some of the devices using a model texturized product. Two specific ingredients, soy protein isolates and insoluble carbohydrates, have been reported by Taranto et al (1978b), Kazemzadeh et al (1982), and Rhee et al (1981) to affect

texture formation of TPP. Therefore, another objective was to examine the similarities between the model extrudates and defatted soy flour extrudates using the results of the texture devices and microscopic techniques.

## MATERIALS AND METHODS

### Extrudate Starting Materials

The soy protein isolate (SPI) used as the protein source was Soya Isolate Promine obtained from Central Soya, Ft. Wayne, IN. The moisture content of the SPI was 10%. Insoluble peanut carbohydrate (IPC) was used as a texture disrupter. IPC was isolated from defatted peanut flour using the method shown in Figure 1. For the mixing and heating of the defatted peanut flour, a typical pilot-plant scale, double-jacket kettle was used. A Westfalia (model SA7-001) automatic desludging, three-phase continuous centrifuge was used for centrifugation. An anhydro, flat-bottom, pilot-plant size spray dryer was used for spray drying.

The IPC and SPI were combined to create mixtures containing 20, 30, 40, 50, 60, 70, and 80% SPI with protein contents of 18.4, 27.6, 36.8, 46, 55.2, 64.4, and 73.6%, respectively (dwb). The SPI was assumed to have a protein content of 92% and the IPC was assumed to have a protein content of less than 1%. The moisture content of each mixture was measured and adjusted to 24% moisture by adding distilled water. Using the Wenger X-5 extruder, each mixture was texturized under identical conditions: raw feed moisture, 24%; steam pressure, 42 kg/ml; process temperature, 150°C; steam lock collar diameter, 20.6 mm; space, 9.5 mm; and screw speed, 650 rpm. Extruded mixtures were dried to 8% moisture and stored at 10°C. Defatted soy flour (Soya Fluff 200 W) was also obtained from Central Soya. In the Food Protein Research and Development Center Analytical Laboratory at Texas A&M University, its proximate composition was determined as: 57.6% protein, 6.7% ash, 1.1% oil, 3.6% crude fiber, 31.0% nitrogen-free extract, and the nitrogen solubility index obtained at pH 6.7-6.8 was 72.0%. These data are very close to the manufacturer's specifications for this product. The moisture content of the soy flour was 8.6% and it was texturized under the same conditions as the SPI-IPC mixtures.

### Specimen Preparation for Microscopic Study

Extruded, texturized products were prepared for transmitted light microscopy (TLM) by the method of Cegla et al (1978). Sections of 0.5-0.75 μm were cut using a Cambridge ultramicrotome and heat fixed to glass slides. The sections were stained and examined by the method of Taranto et al (1978a).

For scanning electron microscopy (SEM), texturized samples were cut perpendicular to their chain long axes and dried in a vacuum oven at 70°C for 24 hr. Samples were fixed on aluminum stubs with silver conducting paint, coated with gold palladium, and examined with a Joel JSM35 scanning electron microscope at a

<sup>1</sup>Product manager, Food Extrusion, Buhler-Miag, Inc., Minneapolis, MN 55440-9497.

<sup>2</sup>Assistant professor, Agricultural Engineering Department, Virginia Tech, Blacksburg 24061.

<sup>3</sup>Professor, Food Protein Research and Development Center, Texas A&M University, College Station 77843.

<sup>4</sup>Associate professor, Institute of Statistics, Texas A&M University.

20° tilt and 25 kV accelerating voltage.

Five photomicrographs of various magnifications were taken of five samples each of the SPI-IPC mixture and soy flour extrudates during each analysis. The photomicrographs presented are representative.

### Mechanical and Physical Measurements

For comparison and method evaluation, texturized products were examined by two mechanical tests and one physical test. The mechanical tests were performed using the Food Technology Corp. CS-1 cell (Kramer type) and the OTMS 10-cm<sup>2</sup> wire extrusion cell. All mechanical measurements were performed using an Instron model 1122 testing machine, operating at a crosshead speed of 50 mm/min.

### OTMS Cell

Samples were ground and separated by sieving on a series of Tyler screens. A particle size range that passed a no. 4 screen but did not pass a no. 6 was saved, and samples were rehydrated with distilled water at a 2:1 ratio for 1 hr at 22° C. Hydrated samples were placed in 250-ml beakers, covered with aluminum foil, and retorted at 121° C for 30 min. Once retorted, samples were cooled in a running tap water bath. Seven and one-half grams of sample was placed in the cell; the force recorded when the plunger had penetrated 1.5 cm was the parameter used as a texture indicator.

### Category 1 Texture Method

The procedure outlined by the USDA Food and Nutrition Service (1974) for the estimation of texture of vegetable protein flour extrudates lacked specificity in many steps. This method was revised, after considerable laboratory experiment, into a standard Category 1 Texture (CIT) method (Meinke 1977) and was used in these experiments.

1) Minus 4 plus 6 mesh textured vegetable protein particles were hydrated for 1 hr at 21–24° C with a water/TPP ratio of 4:1. 2) The

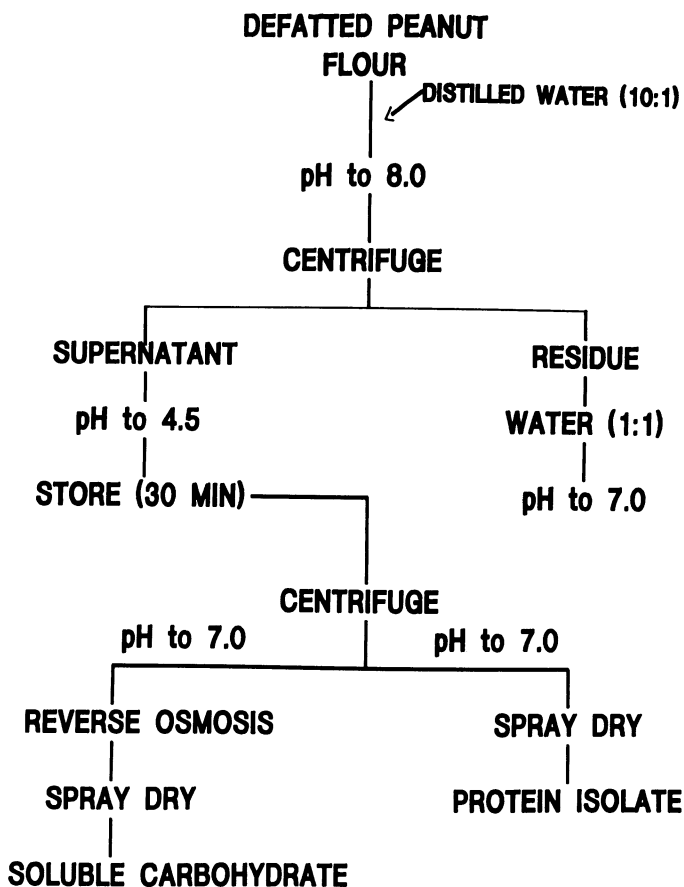


Fig. 1. Flowchart of the method used to separate insoluble peanut carbohydrate.

hydrated sample was retorted for 30 min at 121° C and cooled to 27° C. 3) Excess liquid was drained from the cooled sample on a 40-mesh screen for 4 min. 4) Drained solids were ground through a food grinder fitted with an endplate drilled with 3.2-mm diameter holes. 5) One hundred grams of ground product were weighed onto a 20-mesh screen and spray rinsed 1 min with water at a pressure of 12.5 psig supplied to the spray nozzle and water flow of 119 liters (31½ gallons) per minute. Water was sprayed at a distance of 228 mm (9 in.) from the nozzle face to the 20-mesh screen surface. During the 1-min rinse the screen was rotated (parallel to nozzle face) to assure water spray contact with all sample on the screen. 6) Rinsed solids were drained for 4 min and adhering water was removed from the bottom and outside of the screen with a paper towel. 7) The screen with rinsed solids was weighed. CIT percentage was equal to the weight of rinsed wet solids on the screen when a 100-g sample (step 5) was weighed onto the screen.

### CS-1 Cell

Extrudates were cut into 5-cm lengths and divided into three sets of five samples, three strands each. The first set was dried to 8% moisture after extrusion and designated "dry" samples. The second set was dried to 8% moisture and equilibrated at 87% rh for 24 hr to soften the samples before testing; these samples were designated as "wet I." Samples in the third set (designated wet II) were placed in a 60-ml beaker, which was then placed in a covered 100° C water bath. The samples remained in the resulting steam chamber for 20 min, after which the beakers were immediately covered with

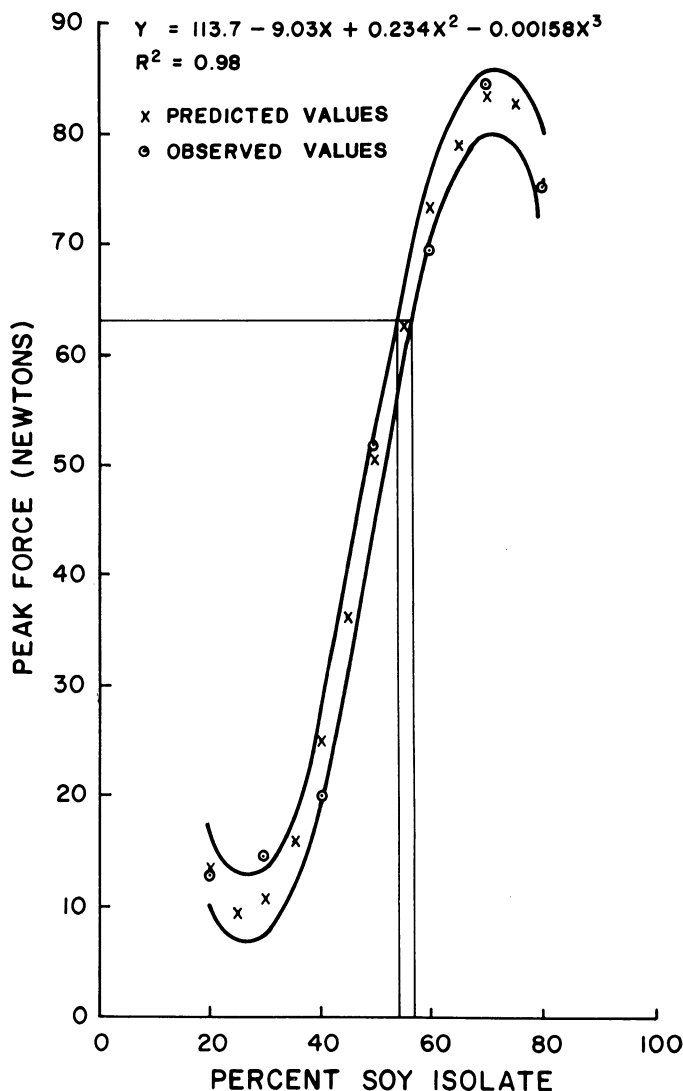


Fig. 2. Peak force (determined with the Ottawa texture measurement system cell) versus percent soy isolate.

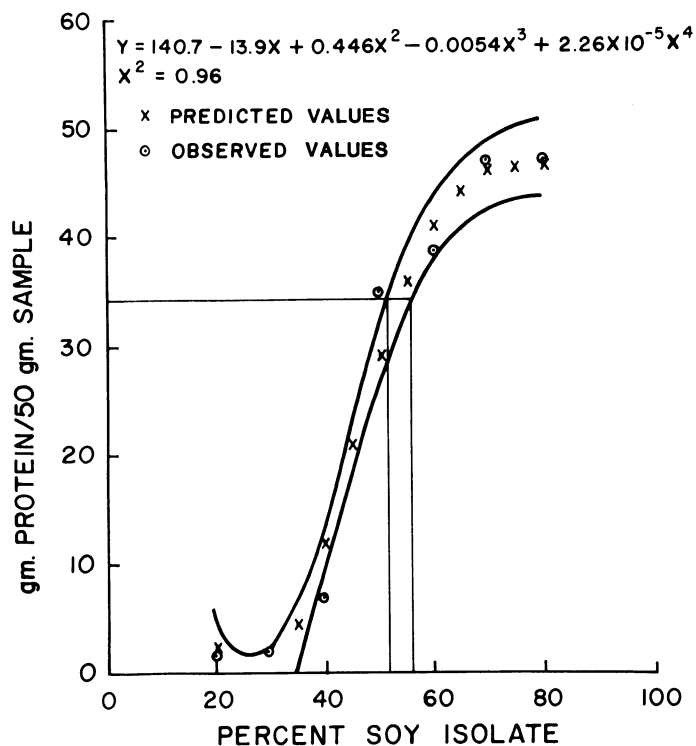


Fig. 3. Category I texture values versus percent protein.

aluminum foil to minimize changes in moisture level on distribution. Samples were cooled to room temperature and tested in the CS-1 cell. The peak force in newtons was used as the texture indicator. Although moisture contents were not determined on all tested samples, moisture contents of like samples were determined to have a range of 37-48%, depending on protein content; lower protein content resulted in lower moisture level.

#### Statistical Evaluation

The data from each method were analyzed by linear or polynomial regressions of the fourth order or less using the Statistical Analysis System (SAS Institute, Cary, NC). The order of the regression was determined by the significance of the regression model parameter estimates. Only those parameters that were significant at the 5% level or less were included in the final regression models. Percent SPI was the independent variable, and the measured values from each evaluation method were the dependent variables. Results obtained from these regressions were considered valid only over the range of SPI-IPC mixtures used.

#### RESULTS AND DISCUSSION

Predicted values (indicated by dots), actual means (dotted circles), and upper and lower 95% confidence limits (smooth curves) obtained from the statistical regressions of the data for the SPI-IPC mixtures are shown in Figures 2-6. Mean values for the physical and mechanical measurements for the soy flour extrudates are indicated on the ordinate axis in Figures 2-6 by a horizontal straight line that crosses the confidence interval band. The confidence intervals are then projected vertically to the

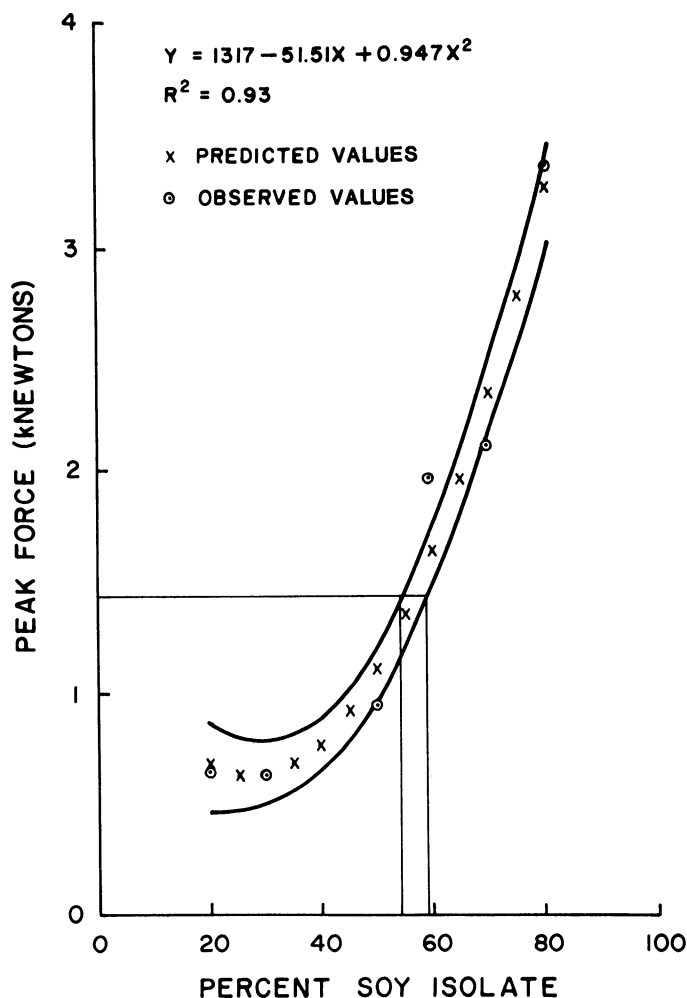


Fig. 4. Peak force (determined with the CS-1 cell) versus percent soy isolate for dry treatment samples.

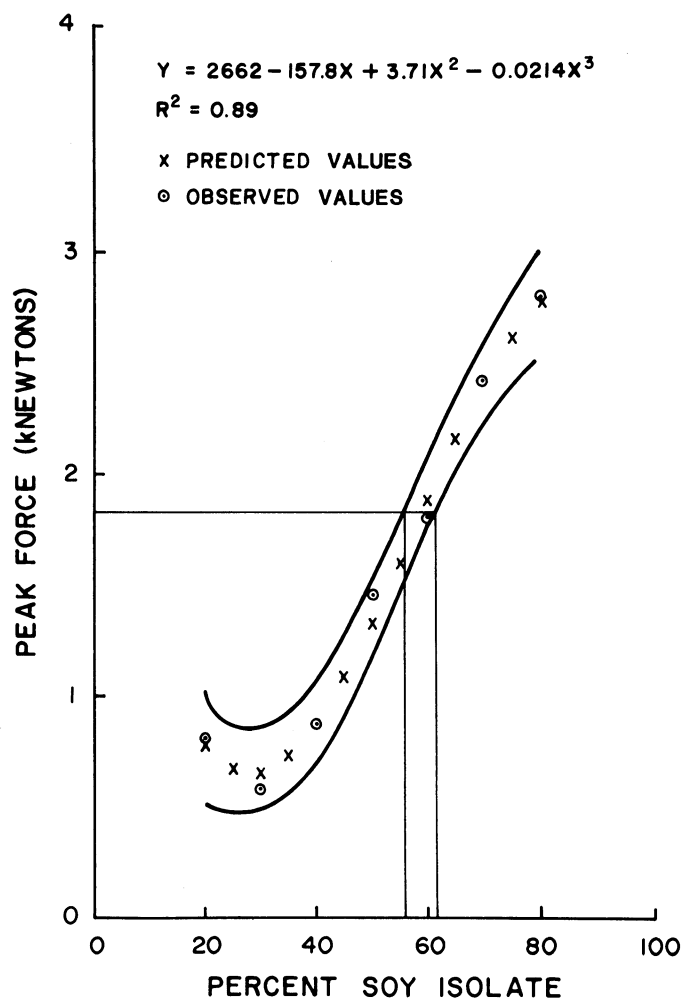


Fig. 5. Peak force (determined with the CS-1 cell) versus percent soy isolate for wet I treatment samples.

abscissa to indicate the confidence interval for comparison with the percent SPI. The data are presented in this manner in order to compare the different texture measurements of the model system extrudates and to quantitatively compare the SPI-IPC and defatted soy flour extrudates. Parameters determined from the texture measurement devices are not intended to be used as predictors of protein content.

#### OTMS Cell

Figure 2 indicates that the regression model yielded a good representation of the data. The mean peak force of the soy flour extrudates indicated an SPI content between approximately 55 and 58% (protein content, 51–53%), which would be lower than the actual value of the soy flour protein content. The data clearly showed a drastic increase in the mechanical behavior of the extrudate as the isolate content increased. Again, the regression model yielded a good representation of the data.

**TABLE I**  
Mean Value for Mechanical and Physical Parameters

Percent Soy Protein Isolate	CS-1 Cell Peak Force (Newtons)			Ottawa Cell Peak Force (Newtons)	Category I Texture (g/50 g)
	Dry	Wet I	Wet II		
	20	655 (16) <sup>a</sup>	805 (8)		
30	626 (15)	583 (12)	1,120 (8)	14.6 (15)	1.8 (27)
40	837 (15)	859 (13)	1,690 (14)	19.8 (17)	6.4 (3)
50	916 (9)	1,440 (15)	2,850 (14)	51.5 (6)	34.5 (5)
60	1,960 (9)	1,810 (15)	2,960 (14)	69.4 (6)	38.6 (5)
70	2,100 (18)	2,390 (9)	4,010 (7)	84.5 (5)	46.4 (8)
80	3,340 (6)	2,790 (23)	4,090 (9)	75.3 (3)	46.5 (9)
Soy flour (control)	1,440 (35)	1,820 (28)	2,940 (9)	63.4 (2)	34.7 (8)

<sup>a</sup>Numbers in parentheses are coefficients of variation.

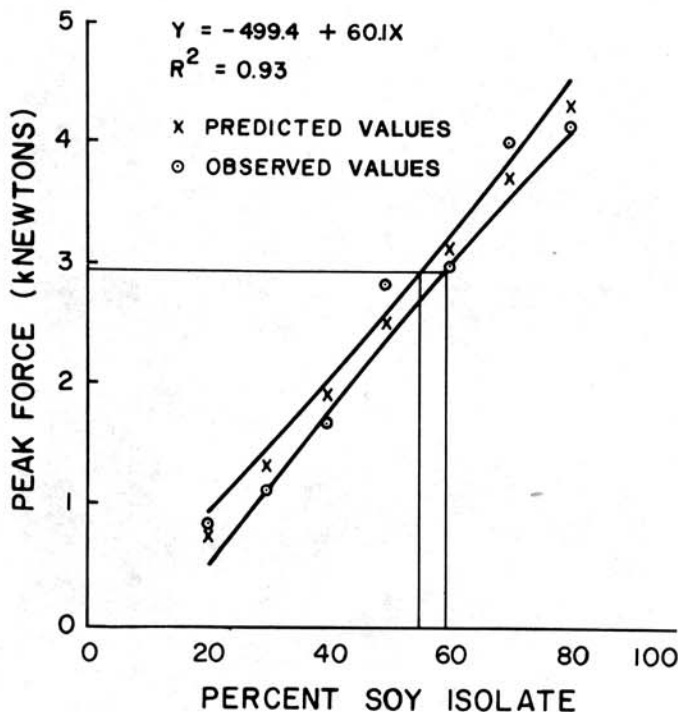


Fig. 6. Peak force (determined with the CS-1 cell) for wet II treatment samples.

#### CIT

Figure 3 contains the results of the CIT method. The drastic increase in this texture parameter is similar to that obtained with the other methods. The SPI range indicated by the measurements on the soy flour extrudate was between approximately 51 and 57% (protein content, 47–52%).

#### CS-1 Cell

Figures 4, 5, and 6 contain the results obtained using the cell CS-1 for the dry, wet I, and wet II samples, respectively. The values are generally highest in wet sample II. This treatment was the most severe in terms of changing moisture content of the extrudates. The samples were moistened in an effort to reduce their brittleness and reduce the variation. The coefficients of variation in Table I indicate that this moistening treatment did consistently reduce variation and also made the extrudates tougher. Other methods of hydrating the extrudates before testing would, no doubt, result in different textural behavior.

Data for the dry sample were well represented by a quadratic equation. The indicated SPI range was between approximately 54 and 59% (protein content, 50–55%). Data for the wet I sample show basically the same trends as those for the dry samples. The indicated SPI range was between approximately 56 and 62% (protein content, 52–57%).

Data for the wet II sample were best represented by a linear regression equation. The indicated range of SPI content was approximately 55–60% (protein content, 51–55%). This would slightly underestimate the soy flour protein content. The linear equation was less ambiguous at the high and low protein contents than the other equations. The slope and intercept were significant at less than the 1% level.

The maxima and minima indicated in Figures 2–6 result from the polynomial regressions used to model the data. Whereas some maxima and minima are shown in the data, these are likely the results of inherent variation. The data in these figures at the extremes of the SPI contents in general show more of a plateau

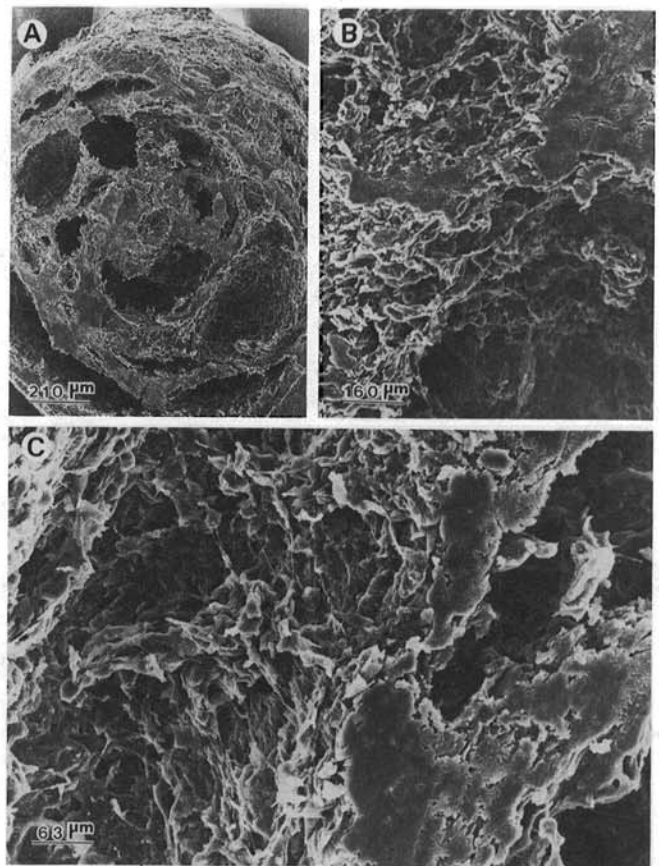


Fig. 7. Scanning electron micrograph of samples containing 20% soy protein isolate and 80% insoluble peanut carbohydrate.

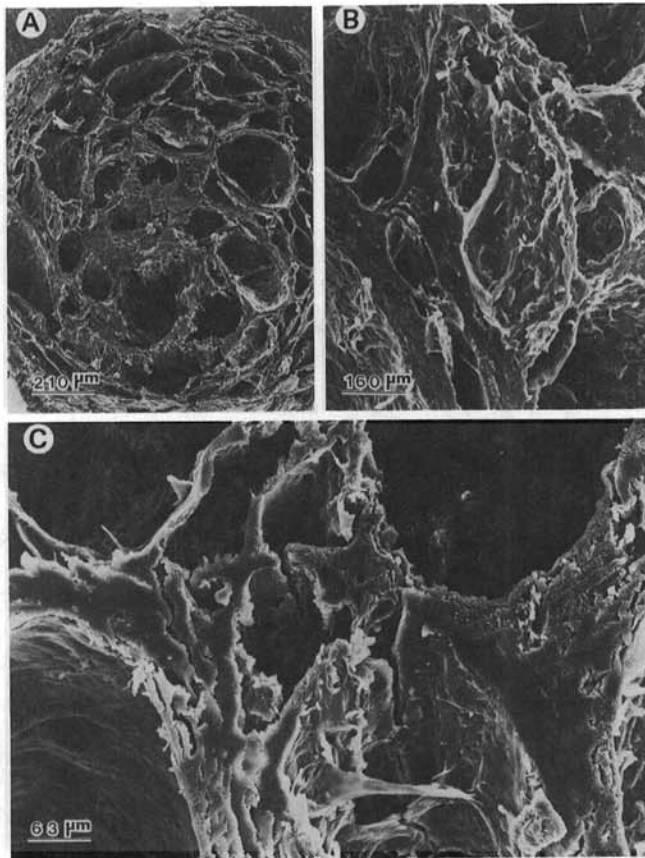


Fig. 8. Scanning electron micrograph of samples containing 40% soy protein isolate and 60% insoluble peanut carbohydrate.

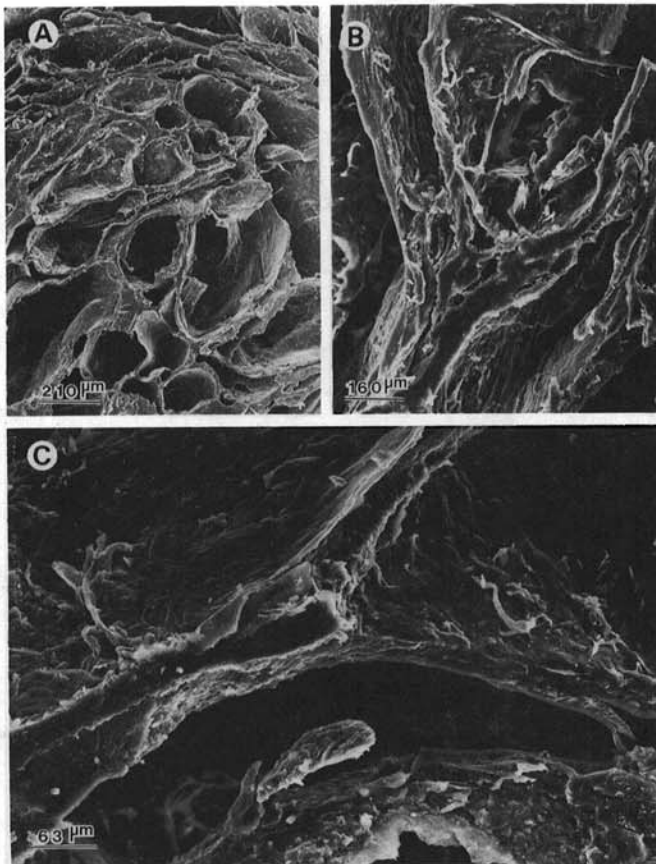


Fig. 9. Scanning electron micrograph of samples containing 50% soy protein isolate and 50% insoluble peanut carbohydrate.

effect. The polynomial regressions were chosen to model the data for simplicity.

All of the tests revealed a pronounced increase in the texture indicators as the SPI content increased from approximately 40 to 70%. This behavior demonstrates the structural integrity provided by protein in TPP. The wet I and II treatments in combination with the CS-I cell in general more closely related the texture and protein content of the SPI-IPC extrudates to that of the defatted soy flour extrudates.

#### SEM

A micrograph of extruded 20% SPI and 80% SPI-IPC carbohydrates is shown in Figure 7. Figure 7A shows the gross morphology of the extrudate in cross section. The cross-sectional diameter is small and the extrudate has irregularly shaped, disrupted air cells. Figure 7B shows highly irregular cell walls, which are barely distinguishable and discontinuous. Air cell surfaces are rough, granular, and flaked (Fig. 7C).

Figure 8 shows a sample of extruded 40% SPI and 60% IPC. Figure 8A presents the gross morphology of a cross section of the extrudate, revealing proportionately more and better defined air cell walls than the 20% SPI extrudate. Figure 8B shows that a typical air cell wall is discontinuous with many disruptions. At higher percent SPI, cell walls are smoother and less flaked on the air surfaces (compare Figs. 7C and 8C).

As the percent SPI in the samples is increased (Figs. 9 and 10), the structure resulting upon extrusion is more porous (Figs. 9A and 10A). At these protein levels (46 and 55.2%), air cells are abundant (Figs. 9B and 10B). Their cell walls are well defined—that is, they stand out clearly from the matrix. For example, Figures 9B and 10B show smoother, more continuous air cells than in 8B. The edges of the air cells tend to be more sharply defined. Figures 9C and 10C show a tendency toward a less wrinkled cell wall with fewer flakes and included granules.

As SPI content is increased to 80% (Fig. 11), extrudate

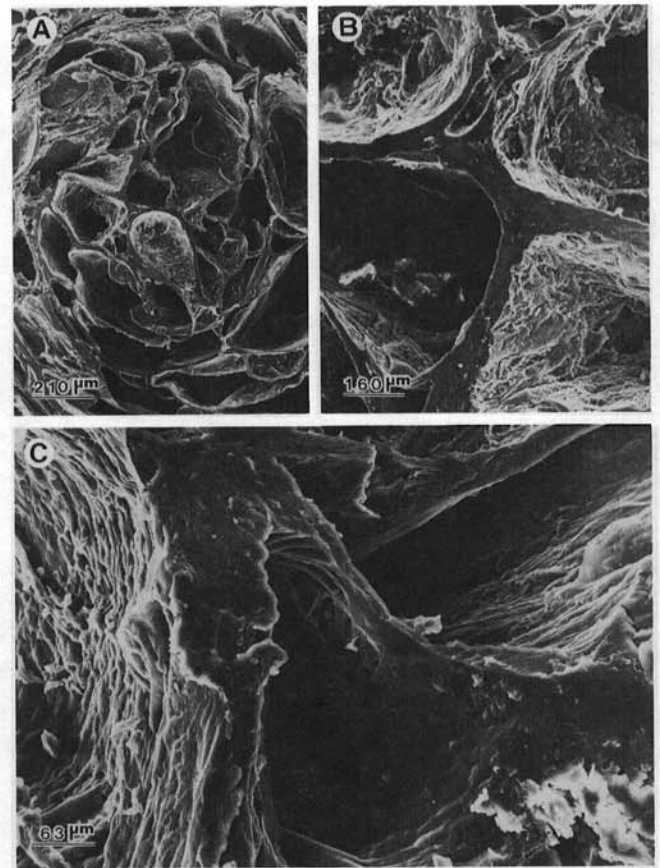


Fig. 10. Scanning electron micrograph of samples containing 60% soy protein isolate and 40% insoluble peanut carbohydrate.



microstructure again changes. In cross section (Fig. 11A), samples have larger air cells and more sharply defined air cell walls. Figure 11B shows a cross section of such an air cell. Its walls tend to be very smooth and continuous, with very sharply defined edges; they are thinner than in previous treatments. In Figure 11C, the appearance of more continuous fibers is seen.

The microstructure of texturized defatted soy flour is shown in Figure 12. The soy flour extrudate was generally less wrinkled and had less subsurface granularity than the SPI-IPC mixtures. In comparing the micrographs of soy flour with those of model SPI-IPC extrudates, the closest resemblances are with Figures 9, 10, and 11, the 50, 60, and 80% SPI samples. When comparing the micrographs at lower magnifications, it is our opinion that the 50 and 60% SPI-IPC mixtures (Figs. 9A and 10A) are most similar to the soy flour extrudates (Fig. 12A). However, comparisons afforded by SEM are subject to the interpretations of the observer, and other observers may find other resemblances in the extrudates.

#### TLM

At an SPI concentration of 20% (Fig. 13A), extrudate microstructure had protein scattered within the IPC matrix. Figure 13B suggests that protein gradually linked with the IPC matrix as SPI concentration is increased to 30%. Figure 13C shows a 40% SPI sample. The protein matrix was not only aggregated, it was becoming continuous. This trend continues (Fig. 13D) at higher SPI concentrations, and in fact, it is possible to see packets of IPC embedded in the protein matrix.

Figure 14A illustrates a continuous protein matrix with insoluble carbohydrate scattered in small quantities throughout that matrix (SPI content, 60%). Figures 14B (70% SPI) and 14C (80% SPI) show slight but continuing trends toward more solid protein matrices with smaller amounts of dispersed IPC.

Figure 14D presents the microstructure of extruded soy flour. It resembles the extruded model mixtures at an SPI concentration of 50–60%. Making comparisons any closer than this would be difficult to justify. However, the formation and consolidation of the protein matrix as the protein content increases was readily apparent.

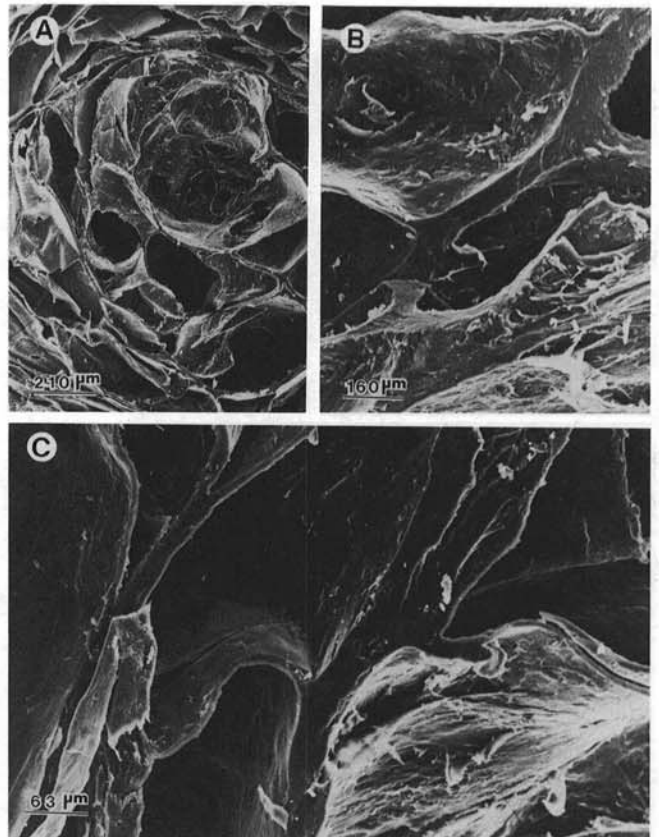


Fig. 12. Scanning electron micrograph of texturized defatted soy flour sample used as control.

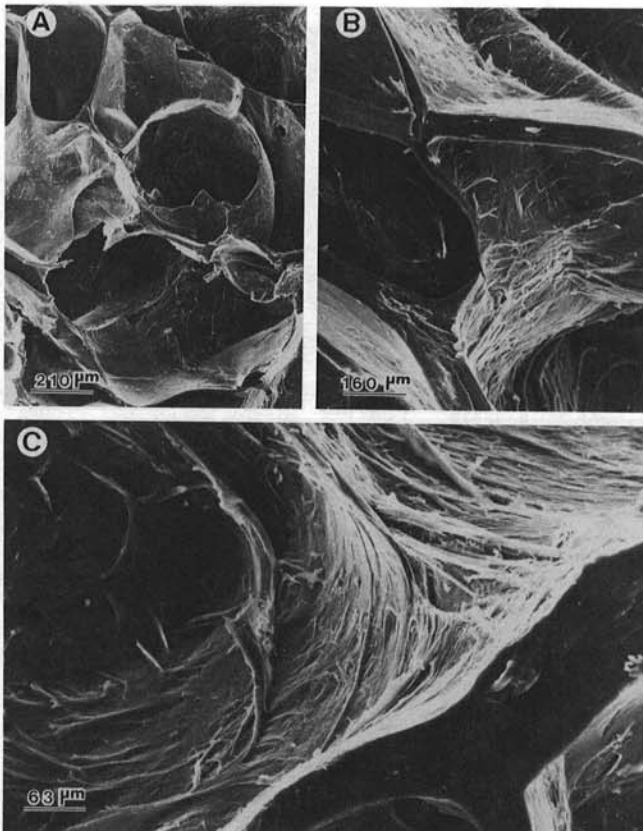


Fig. 11. Scanning electron micrograph of samples containing 80% soy protein isolate and 20% insoluble peanut carbohydrate.

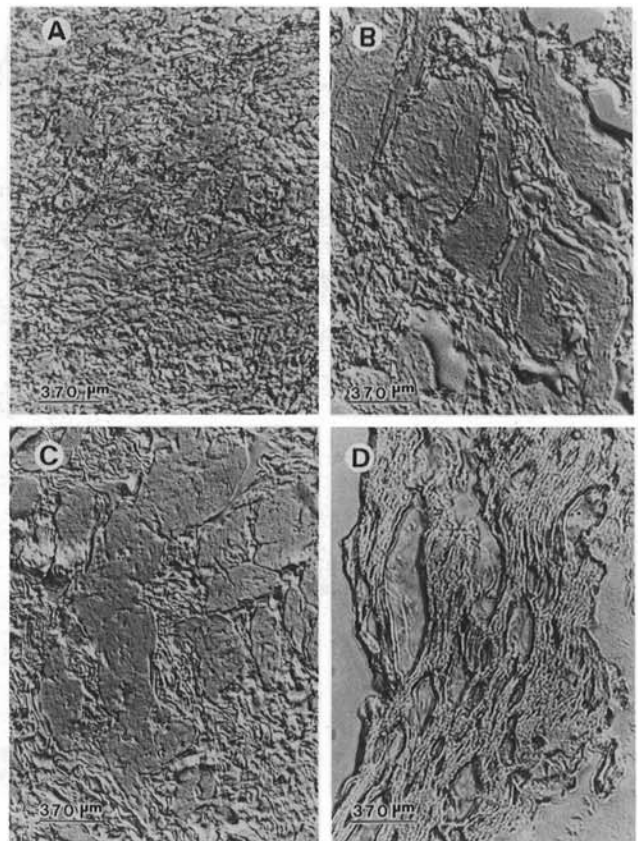


Fig. 13. Transmitted light micrograph of samples containing A, 20%; B, 30%; C, 40%; and D, 50% soy protein isolate.

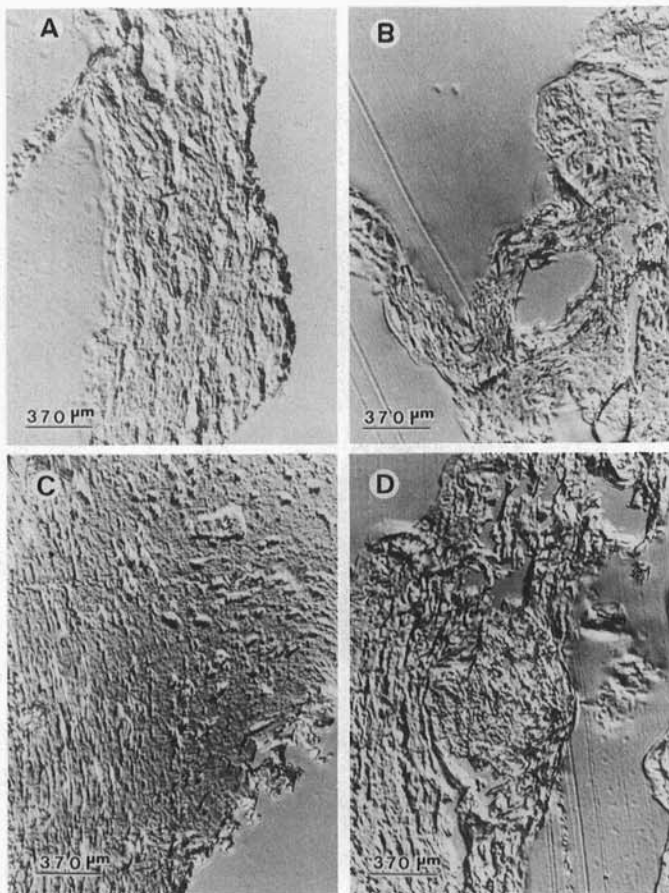


Fig. 14. Transmitted light micrograph of samples containing A, 60%; B, 70%; C, 80% soy isolate; and D, defatted soy flour control.

## CONCLUSIONS

Most of the nonmicroscopic evaluations used in these studies yielded an increase in magnitude of the recorded parameters as the protein in the extrudate increased. This supports the hypothesis that protein is essential if not responsible for a well-developed texture in soy-based mixtures. This is probably because proteins contribute to the skeletal structure of the texturized product in which the carbohydrates are dispersed. Observations by SEM and TLM provided additional support for this hypothesis.

Of the physical and mechanical tests used to evaluate the extrudates, the wet I and II sample preparation used with the CS-I cell most closely related the textural behavior and protein content of the SPI-IPC mixtures to that of the soy flour extrudate. The sample preparation was also simpler for these treatments than for the other tests. These results proved useful in demonstrating the role of protein presence and level in mechanical response and structure formation.

As with other extrusion research, results cannot be assumed to be translatable to other raw material compositions, extruder types or processing parameters; however, the trends shown above were both consistent and logical. The comparisons indicated that the CS-I cell used in combination with a consistent and reliable preparation technique may provide a useful alternative to the CIT

method for evaluating the effects of processing treatments on extrudate quality. Analysis of ingredients of different compositions, processed in different extruders under different processing conditions, would be of great benefit in broadening the application of these results or in establishing a common basis for physical comparison of extrudates. Because the insoluble carbohydrate was purified manually, the quantity of material obtained was sufficient for one extruder run only. For this reason these results should be viewed in a relative sense only.

Steam flashing from the extrudate as it exited the extruder die provided the extrudate with its characteristic porous structure. However, the micrographs presented here indicated that this porous structure will not develop properly unless a minimum level of protein is present. Mechanical and microscopic comparisons of the soy flour extrudates with the SPI-IPC mixtures suggested this level to be in the range of 50–60%. For a prescribed set of processing conditions, other chemical factors could alter the level of protein needed for proper textural and structural formation. The difference, if any, in basic thermal and rheological properties between various SPI-IPC mixtures could alter the quantity of real thermal processing that the mixtures received even though the extruder processing conditions were held constant. This possibility could affect proper structural development. Such effects will be difficult to show until more properties data and realistic equations that model heat and flow profiles in extruders are developed.

## LITERATURE CITED

- BREENE, W. M., and BARKER, T. G. 1975. Development and application of a texture measurement procedure for textured vegetable protein. *J. Texture Stud.* 6:459-472.
- CEGLA, G. F., TARANTO, M. V., BELL, K. R., and RHEE, K. C. 1978. Microscopic structure of textured cottonseed flour blends. *J. Food Sci.* 43:152.
- CUMMING, D. B., STANLEY, D. W., and DeMAN, J. M. 1972. Texture structure relationships in texturized soy protein II. Textural properties and ultrastructure of rehydrated spun soy fibers. *Can. Inst. Food Sci. Tech. J.* 5(3):124.
- FOOD AND NUTRITION SERVICE. 1974. National school lunch program: Special food service program for children. *Fed. Regist.* 39(60):1197.
- KAZEMZADEH, M. 1980. Morphological and ultrastructural study of extrusion texturized defatted soy flour. M.S. thesis. Texas A&M University, College Station, TX.
- KAZEMZADEH, M., AGUILERA, J. M., and RHEE, K. C. 1982. Use of microscopy in the study of vegetable protein texturization. *Food Technol.* 36(4):111.
- LOH, J., and BREENE, W. M. 1977. Texture analysis of textured soy protein products: Relations between instrumental and sensory evaluation. *J. Texture Stud.* 7:405-419.
- MAURICE, T. J., and STANLEY, D. W. 1978. Texture-structure relationships in texturized soy protein IV. Influence of process variables in extrusion texturization. *Can. Inst. Food Sci. Tech. J.* 11(1):1-6.
- MEINKE, W. W. 1977. Final report on textural vegetable protein from cottonseed flours. Research Agreement 12-14-7001-281. SRR-SEA USDA: New Orleans, LA.
- RHEE, K. C., KUO, C. M., and LUSAS, E. W. 1981. Texturization. Page 51 in: *Protein Functionality in Foods*. ACS Symp. Ser. 147. J. P. Cherry, ed. Am. Chem. Soc.: Washington, DC.
- TARANTO, M. V., CEGLA, G. F., BELL, K. R., and RHEE, K. C. 1978a. Texturized cottonseed and soy flours. A microscopic analysis. *J. Food Sci.* 43:767-771.
- TARANTO, M. V., CEGLA, G. F., and RHEE, K. C. 1978b. Morphological, ultrastructural and rheological evaluation of soy and cottonseed flours texturized by extrusion and non-extrusion processing. *J. Food Sci.* 43:973-979, 984.

[Received June 24, 1985. Accepted December 2, 1985.]