Functional Properties of Low-Fat Soy Flour Produced by an Extrusion-Expelling System

A.A. Heywood[a], D.J. Myers[a,*], T.B. Bailey[b], and L.A. Johnson[a]

[a]Department of Food Science and Human Nutrition and Center for Crops Utilization Research, and [b]Department of Statistics, Iowa State University, Ames, Iowa 50011

ABSTRACT: Low-fat soy flour (LFSF) obtained by extrusion-expelling processing was investigated for functional properties. Flours with the following various levels of protein dispersibility indexes (PDI) and residual oil (RO) contents were investigated: "high" 67 ± 4/10.4 ± 1, "mid" 42 ± 3/7.4 ± 2, and "low" 14 ± 5/6.5 ± 0. The solubility of all three LFSF was minimal at pH 4.0 and increased at more alkaline and acidic pH levels. Water-holding capacity (WHC) increased with a decrease in PDI and RO content, whereas fat-binding capacity (FBC) decreased. Foaming stability increased as PDI and RO increased, with significant differences between all LFSF samples. Emulsification capacity (EC) was measured at three pH levels (5.5, 6.7, and 8.0). At each pH level, the "low" samples showed the least EC compared to the "mid" and "high" samples, with no significant difference between the "mid" and "high" samples at pH 6.7 and 8.0. Emulsification stability and activity decreased from low LFSF to high LFSF. This study showed that in general low LFSF was less functional than the other flours tested and there was no significant difference in the functionality of mid- and high-LFSF samples.

Paper no. J10123 in *JAOCS 79,* 1249–1253 (December 2002).

KEY WORDS: Extrusion-expelling systems, functional properties, low-fat soy flour, soybean protein.

Research efforts to find value-added uses for soybean protein in food and nonfood products have been going for some time. Researchers have found that soy protein is a promising substitute for animal protein in foods because it contains all of the essential amino acids required for the diet, does not negatively affect sensory attributes (except flavor), and has additional health benefits including the lowering of blood cholesterol levels and reduction of risk of some cancers (1,2). In nonfood applications, soy protein has been used in wood adhesives as a partial replacement for petroleum-based ingredients, and in other applications such as biodegradable plastics and paper coatings and sizings (3). These value-added uses for soy proteins are based on the functional properties of the protein that add key characteristics to the food or nonfood product that is being formulated. The properties include emulsification, foaming, gelation, and water and fat binding. Recently, researchers have sought to add additional value to soy protein by using alternative processing techniques, genetic engineering, or traditional plant breeding to incorporate new, desirable characteristics or alter undesirable characteristics inherent in the soybean. In this paper, the potential for adding value to soy through an alternative processing technique, namely extrusion-expelling (EE) processing, will be discussed.

Traditional industrial soybean processing involves solvent extraction of the oil with subsequent desolventizing and drying of the meal. The meal is then further processed *via* grinding and separation steps to produce flour, meal, or grits. An alternative soybean processing technique is the EE process developed by Nelson *et al.* (4) at the University of Illinois. EE processing relies on the mechanical extraction of soybean oil and thus does not incorporate any chemicals in the extraction process. The meal remaining can then be processed in a manner that produces products similar to those from traditional soybean processing, i.e., meal and flour. EE equipment produces low-fat soy flour (LFSF). LFSF is defined as having 5 to 6% residual fat (5). A significant number of small soybean processors, those that process between 6 and 120 tons of soybeans per day, utilize this technology because of the low capital investment costs, enhanced extraction capabilities, and the ability to produce oxidatively stable oils and meals low in FFA (4).

To begin the EE process, soybeans are dried, dehulled, and cracked. These beans then enter an extruder, which has a variety of restrictions, thus producing heat to inactivate antinutritional factors. Upon exiting the extruder, the soybeans are in a semisolid state. This mixture then enters the expeller, where the oil is pressed out and the meal ejected in large, solid pieces. A mill (generally a roller mill) is used to grind these large pieces into smaller particles that may either be consumed (as animal feed) or be further ground into flour for human consumption (4).

Some researchers have hypothesized that EE meal may have additional functional characteristics over defatted soybean meal due to the approximately 5 to 6% residual oil: Defatted soybean meal contains less than 0.5% oil. Functional characteristics are properties that promote improved behavior in food systems and industrial applications of protein and include the protein's solubility, water-holding capacitites (WHC) and fat-binding capacities (FBC), emulsification properties, and interactions with hydrophilic and hydrophobic materials. However, limited research has been published on the functional characteristics of LFSF coming from the EE process.

The objective of this study was to characterize the LFSF produced from the EE processing system in terms of the fol-

*To whom correspondence should be addressed at Iowa State University, 2312 Food Sciences Bldg., Ames, IA 50011. E-mail: dmyers@iastate.edu

lowing functional properties: protein solubility profile, foaming and emulsification characteristics, and WHC and FBC.

EXPERIMENTAL PROCEDURES

Preparation of LFSF. Low-fat soy meal was processed at Iowa Soy Specialties (Vinton, IA). This meal was then taken to the Center for Crops Utilization Research Center (CCUR) at Iowa State University where it was milled into flour on a hammer mill (Fitzpatrick Company, Elmhurst, IL) to approximately 100 U.S. mesh size. The processing parameters used are described in detail in the study by Crowe (6). The flours produced were categorized into three protein dispersibility index/residual oil (PDI/RO) groupings: low LFSF, 14 ± 5/6.5 ± 0; mid LFSF, 42 ± 3/7.4 ± 2; and high LFSF, 67 ± 4/10.4 ± 1.

Proximate analysis. Proximate analyses for crude protein (PerkinElmer Series II Nitrogen Analyzer), moisture (AOCS Ba-38) (7), fat (AACC 30-25) (8), and ash (AOAC 942.05) (9) were performed. An outside laboratory (Woodson-Tenent, Des Moines, IA) performed the analyses for PDI by using the fast-stir method (AOCS Ba 10-65) (7).

Solubility. In a 50-mL centrifuge tube, a sample weighing 250 mg was dispersed in 25 mL of distilled water. This solution was adjusted to the appropriate pH with 1 N HCl or NaOH, shaken at 120 rpm (VersaBath S model 224, Fisher Scientific) at 25°C, and centrifuged (Sorvall RC 5 Plus) at 30,597 × *g* for 30 min (10). The resulting supernatant was filtered through Whatman No.1 filter paper, and nitrogen determination was performed on 10 mL of the filtered supernatant following Kjeldahl procedures. Protein solubility was calculated using the following:

$$\text{protein solubility (\%)} = \frac{\text{supernatant protein concentration (mg/mL)} \times 25}{\text{sample wt (mg)} \times [\text{sample protein content}/100]} \times 100 \quad [1]$$

Emulsification capacity (EC). A modified version of the method of McWatters and Holmes (11) was followed. A 2% protein suspension in water was prepared. The pH of this suspension was altered to either 5.5, not altered (natural pH used), or 8.0 with 1 N NaOH or 1 N HCl in order to observe the effect of pH on EC. A 2% protein suspension (25 mL) was placed in a 500-mL plastic beaker. The suspension was continuously blended with a hand-held mixer (Bamix, Mettlen, Switzerland) at high speed (approximately 12,000 rpm) with soybean oil at a flow rate of 1 g/s. This mixture was continuously blended until the inversion point (oil-in-water) was visually observed. EC was determined as the maximum amount of oil emulsified on a per gram protein basis.

Emulsification activity index (EAI) and stability index (ESI). A 2% protein suspension (21 mL) was blended with 7 mL of soybean oil for 1 min using a Waring blender with a microcontainer (110 mL size; Fisher Scientific) at low speed. This emulsion was immediately diluted with 0.1% SDS at a 500× dilution factor, and the absorbance measured at 500 nm (Shimadzu Spectrophotometer, UV-160). The diluted emulsion was then incubated at 95°C in a water bath (Fisher Scientific) and the absorbance of the emulsion measured at time zero and after 10 min. The EAI and ESI were calculated using the absorbance measured at time zero (A_0) and at 10 min (A_{10}). Calculations as defined by Pearce and Kinsella (12) were used to calculate EAI and ESI:

$$\text{EAI (m}^2\text{/g)} = 2T/\Phi C \quad [2]$$

where C = weight of protein per unit volume of aqueous phase before emulsion is formed; $T = 2.303A/l$ (A = absorbance, l = pathlength of cuvette); and $\Phi = C - A - E(B - C)/C - A + (B - C)$ $[(1 + E)D_o/D_s - E]$ where A = mass of beaker, B = mass of beaker plus emulsion; C = mass of beaker plus dry matter; D_o = density of oil; D_s = density of protein solution; and E = concentration of solutes (mass per unit mass of solvent). Also,

$$\text{ESI (min)} = A_0 \times \Delta t / \Delta A \quad [3]$$

where Δt = 10 min and $\Delta A = A_0 - A_{10}$.

Foaming capacity (FC). A 0.5% protein suspension (80 mL) was added to a glass column with a fritted glass disk (medium pore size) on the bottom. Nitrogen gas was purged through the column at a flow rate of 100 mL/min. FC and foaming stability (FS) were calculated based on the equations described by Sorgentini *et al.* (13):

$$\text{FC} = V_f\,(\text{mL})/f_r\,(\text{mL/min}) \times t_f\,(\text{min}) \quad [4]$$

where V_f = fixed volume of foam, 150 mL; f_r = flow rate of N_2 gas, 100 mL/min; t_f = time to reach fixed foam volume.

$$\text{FS: } K = 1/V_{\max} \times t_{1/2}\,(\text{mL}^{-1}\,\text{min}^{-1}) \quad [5]$$

where $V_{\max}$ = volume of liquid incorporated in foam at V_f; $t_{1/2}$ = time to drain half of the liquid incorporated into foam.

WHC. Methods modified from Lin and Zayas (14) were used to determine WHC. LFSF (5 g) was weighed and dispersed into 95 mL of distilled water and mixed with a magnetic stir bar for 20 min at 25°C. Three 50-mL centrifuge tubes were filled with the flour/water solution and centrifuged at 1,074 × *g* for 30 min. After disposing of the supernatant, the WHC was calculated as the difference between the weight of the hydrated flour and the weight of the centrifuge tubes. WHC was expressed as grams of water per gram of protein.

FBC. The FBC was determined by stirring a 5% soy flour solution with 50 mL of corn oil (Hy-Vee Brand, West Des Moines, IA) for 30 min and then allowing it to stand for 30 min at room temperature (25°C). This mixture was then placed into two 50-mL centrifuge tubes and centrifuged for 30 min at 1,074 × *g*. After disposing of the excess oil, the FBC was calculated as the RO divided by the original weight (15). FBC was expressed as grams of oil per gram of protein.

Data analysis. Production of soy flour followed a completely randomized design. Functionality tests were carried out using a completely randomized block design with the exception of EC. A factorial design was utilized to analyze data from the EC functionality test. The general linear model procedure was used to analyze all functionality tests. Tukey was used for multiple comparisons, and significance was determined at the $P < 0.05$ level. Statistical analysis was carried

out using SAS statistical software (SAS Institute Inc., Version 8.0, Cary, NC).

RESULTS AND DISCUSSION

Proximate analysis. Table 1 shows the proximate compositions of all flours used in this experiment. One note that should be made is that during processing, the objective of the researchers was to obtain flour in three distinct PDI/RO ranges. However, based on equipment capabilities, the amount of material that was obtained in each category was unequal. Thus, we have two flours that comprise low LFSF, seven flours that comprise mid LFSF, and six that comprise high LFSF. One commercially available defatted soy flour (DFSF) was used as the control. Results show that the moisture content decreases with PDI in the LFSF. This is due to increased heating of these low-PDI flours, thus allowing for more moisture to be driven off. The protein is lower in the LFSF than in the DFSF. This trend deviates slightly from published values for each soy flour (5). Carbohydrate content was calculated by difference. These values are slightly lower than typical carbohydrate contents of soy flour (5). The PDI is an indirect measure of the amount of heat treatment applied to each soy flour; the more heat treatment, the lower the PDI. The PDI measurement has also been found to correlate with protein functionality (16): When there is a decrease in PDI, there is a decrease in functionality. When EE processing is used, the fat content is correlated to the PDI and thus to the heat treatment. The preconditioning step (extrusion) is used to disrupt the spherosomes, thus allowing more oil to be expelled (4). When a less intense heat treatment (or lowering of time spent in extruder) is used, the degree of tissue disruption is decreased and the amount of oil retained in the soy meal is increased.

Protein solubility. The protein solubility curve is shown in Figure 1. All three LFSF and the DFSF show minimal solubility at pH 4.0 and an increase in solubility at increased alkaline and acidic pH levels. These curves indicate that the protein solubility of the flour is affected by the degree of heat treatment and pH. The low LFSF received the most heat treatment, whereas the high LFSF received the least. The results for the high LFSF and DFSF show that these two products are relatively equal in solubility at pH 8.0.

TABLE 1
Proximate Composition of Low-Fat Soy Flours (LFSF) and Defatted Soy Flour (DFSF)[a]

Component (%)	High LFSF[b]	Mid LFSF[c]	Low LFSF[d]	DFSF[e]
Moisture	6.9	5.6	4.1	9.4
Crude protein	49.6	50.9	50.2	53.2
Fat[f]	10.4	7.4	6.5	<0.5
Ash	5.7	5.9	6.0	5.0
Carbohydrate[g]	26.4	30.3	33.2	32.5
PDI	66.6	41.6	14.3	69.8

[a]Results are expressed on a dry weight basis.
[b]Mean of six flours.
[c]Mean of seven flours.
[d]Mean of two flours.
[e]ADM Bakers Nutrisoy (Decatur, IL).
[f]Ether extract.
[g]By difference. PDI, protein dispersibility index.

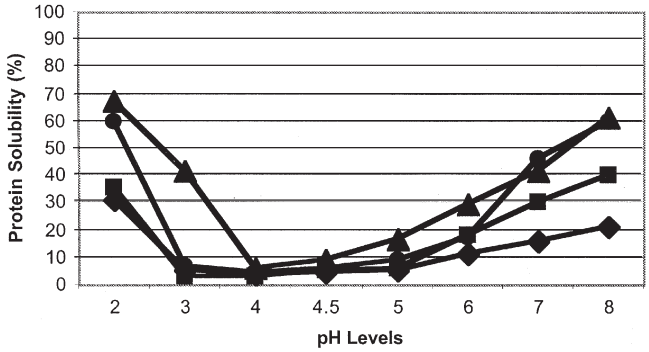

FIG. 1. Protein solubility curves for low-fat soy flour (LFSF) and defatted soy flour (DFSF). ◆, Low LFSF; ■, mid LFSF; ●, high LFSF; ▲, DFSF.

Protein solubility is considered to be one of the most important functionality tests because it is an indication of how the protein will perform in other functionality tests (17). The PDI is related to the solubility of the protein. Thus, the higher the PDI, the more soluble a protein is. Furthermore, the solubility of a protein may indicate how useful this protein will be in food systems. Therefore, mid–high LFSF and DFSF would be more functional than low LFSF in a food system based on solubility.

EC. EC is defined as the maximal amount of oil that is emulsified by a protein dispersion (16). The EC for all samples is shown in Figure 2. The EC data show an increase in EC with an increase in pH and PDI/RO. McWatters and Holmes (11) state that EC is affected by protein solubility. As a protein approaches the specific isoelectric point, there is a decrease in net electrical charge and thus minimal solubility and reactivity are found (18). In this system at pH 5.5, proteins are less soluble and therefore have a decreased capacity to act as surface-active agents and absorb at the oil/water interface. This

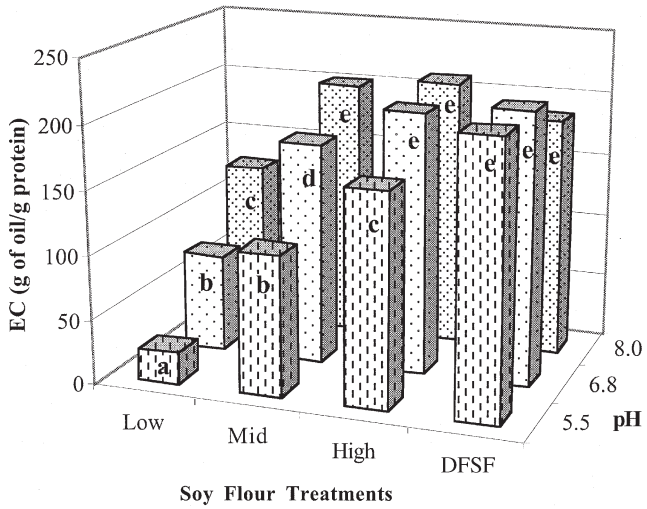

FIG. 2. Emulsification capacity (EC) of LFSF and DFSF. Bars with different letters are significantly different at $P < 0.05$. For other abbreviations see Figure 1.

decrease in surface activity leads to a decrease in EC. Among low, mid, and high LFSF, as the PDI/RO increases there is also an increase in EC. These data suggest that in the protein samples with the less denatured protein, as indicated by PDI, the EC increases. Another hypothesis is that RO may play a part in these EC results. As the RO content increases, the hydrophobicity of the protein increases and in turn allows a greater amount of oil to be emulsified.

Significant differences were found between all soy flours at pH 5.5. However, at pH 6.7 and 8.0, the only significant differences found are those between the low LFSF and all other soy flour samples. There is no significant difference between the defatted soy flour and mid- and high-LFSF samples.

The viscosity of these emulsions was not measured. However, emulsions that resulted in an EC of less than 100 (g oil/g protein) could be considered simply a suspension, not an emulsion, due to the extremely low viscosity. The inversion point of these emulsions was difficult to identify due to this very low viscosity.

Emulsification activity and stability. The EAI is a measure of the area of interface that is stabilized per unit weight of protein, whereas the ESI is a measure of the emulsion's resistance to breakdown (12). The EAI was the highest in low LFSF and lowest in the DFSF (Table 2). There was no significant difference between mid–high LFSF and DFSF. The ESI showed the same trend as EAI with a decreased ESI when moving from low to mid and high LFSF to DFSF; again, there was no significant difference between the mid–high LFSF and the DFSF. These results indicate that the low LFSF shows more activity and stability in emulsions yet decreased EC compared to the rest of the flour samples. One hypothesis that may explain these results is that low LFSF may have more exposed hydrophobic regions owing to the denaturation that has occurred during processing and may react with the lipids present forming "lipoprotein-like" materials that have surface activity; thus, the low LFSF has an increased ESI and EAI compared to the other LFSF and DFSF.

WHC and FBC. The WHC and FBC results are shown in Table 3. RO is hypothesized to play a role in both of these tests. WHC shows a significant decrease for the high LFSF. Comparing the high LFSF data with the DFSF results shows a significant difference in the two readings, although the PDI readings are very similar. This result could be attributed to the amount of RO present in high LFSF sample. The mean RO for the high PDI-LFSF samples is 11%, much higher than the DFSF (<0.5%). The presence of this additional fat (a hydrophobic material) could result in less available hydrophilic binding sites available for water holding by the protein.

TABLE 3
Water-Holding Capacity (WHC) and Fat-Binding Capacity (FBC) of LFSF and DFSF

Treatment	WHC[a]	FBC[a]
Low LFSF	6.75[a]	1.66[b]
Mid LFSF	6.19[a]	1.74[b]
High LFSF	4.79[b]	1.84[b]
DFSF	6.70[a]	2.22[a]

[a]WHC and FBC in g water (oil) per gram of protein. Means with different superscripts are significantly different at $P < 0.05$. For abbreviations see Table 1.

Fat absorption capacity results are also shown in Table 3. The data show that the DFSF had a much greater degree of FBC than any of the LFSF samples. The mechanism for fat absorption by soy protein has not been elucidated although fat absorption is commonly attributed to the physical entrapment of fat by the protein (18). Thus, it can be theorized that the residual fat that is present in LFSF is blocking the hydrophobic binding sites usually available for binding hydrophobic substances. The DFSF theoretically has all the hydrophobic binding sites available for uptake of hydrophobic materials. It is thought that the greater the amount of heat treatment that is given to a protein, the more hydrophobic the protein becomes as a result of a greater number of hydrophobic groups being exposed through the unfolding of the protein's 3-D structure. The results obtained from this study show a trend that deviates from this accepted theory; however, the results obtained here agree with results by Hutton and Campbell (19) which showed that soy protein decreases in fat absorption capacity with increased heat.

FC and FS. The FC and FS results are shown in Table 4. FC is a measure of the maximum level of foam generated by a solution, whereas foaming stability is a measure of the resistance of the foam to destabilization (10). The amount of foam that a protein can produce is important, but more important is the stability of the foam. Thus, although the FC data are presented, the FS data will be focused on. FS data are interpreted as: The lower the value, the more stable the foam. The data show a very large variation in FS between the DFSF and LFSF. The DFSF produced very stable foams, with symmetrical, evenly distributed foam bubbles. The size of the bubbles is significant because this is an indication of stability

TABLE 2
Emulsification Activity and Stability

Treatment	EAI[a] (m^2g^{-1})	ESI[b] (min)
Low LFSF	15.36[a]	12.78[a]
Mid LFSF	12.09[b]	11.35[b]
High LFSF	11.21[b]	10.28[c]
DFSF	10.77[b]	10.36b[c]

[a]Emulsification activity index (EAI).
[b]Emulsification stability index (ESI). Means with different superscripts are significantly different at $P < 0.05$. See Table 1 for other abbreviations.

TABLE 4
Foaming Properties of LFSF and DFSF

Treatment	Foaming capacity[a]	Foaming stability[b]
Low LFSF	0.81[c]	0.37[a]
Mid LFSF	0.85[a]	0.14[b]
High LFSF	0.88[b]	0.11[c]
DFSF	0.85[a]	0.01[d]

[a]mL of foam/mL of $N_2 \times$ min.
[b]$mL^{-1} \times min^{-1}$. Means with different superscripts are significantly different at $P < 0.05$. For abbreviations see Table 1.

(17). As the data would suggest, the less stable the foam, the larger the bubbles. As with WHC and FBC, the foaming properties of EE LFSF are dependent not only on the PDI of the flour but also could be influenced by the RO content. Morr (20) states that hydrophobicity enhances FS. Thus, these results could again suggest that the hydrophobicity of these LFSF is increased. When the FS of LFSF is compared to the FS of DFSF, the potential interfering effect of RO is demonstrated by the decreased FS of the LFSF samples, particularly in the case of the high LFSF, which has a PDI relatively equal to DFSF.

ACKNOWLEDGMENTS

Funding for this research was provided by the Iowa Soybean Promotion Board in conjunction with the Center for Crops Utilization Research at Iowa State University. The authors would like to thank Troy Crowe and Dr. Tong Wang for their assistance in processing of soybeans, and Troy Crowe for performing proximate analyses.

REFERENCES

1. Riaz, M.N., Soybeans as Functional Foods, *Cereal Foods World 44*:88–92 (1999).
2. Arditi, T., T. Meredith, and P. Flowerman, Renewed Interest in Soy Isoflavones and Saponins*, Ibid. 45*:414–417 (2000).
3. Johnson, L.A., D.J. Myers, and D.J. Burden, Early Uses of Soy Protein in Far East, U.S., *inform 3*:282–290 (1992).
4. Nelson, A.I., W.B. Wijeratne, S.W. Yeh , T.M. Wei, and L.S. Wei, Dry Extrusion as an Aid to Mechanical Expelling of Oil from Soybeans. *J. Am. Oil Chem. Soc. 64*:1341–1347 (1987).
5. Golbitz, P., *Soya and Oilseed Bluebook,* Soyatech, Bar Harbor, ME, 2001, pp. 377, 390.
6. Crowe, T.W., and L.A. Johnson, Twin-Screw Extrusion Texturization of Extruded-Expelled Soybean Flour, *J. Am. Oil Chem. Soc. 78,* 781–786 (2001).
7. AOCS, *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 4th edn., AOCS Press, Champaign, 1993.
8. AACC, *Approved Methods of the American Association of Cereal Chemists*, 8th edn., American Association of Cereal Chemists, St. Paul, MN, 1983.
9. AOAC, *Official Methods of Analysis*, 15th edn., Association of Official Analytical Chemists, Gaithersburg, MD, 1990.
10. Morr, C.V., B. German, J.E. Kinsella, J.M. Regenstein, J.P. Van Buren, A. Kilara, B.A. Lewis, and M.E. Mangino, A Collaborative Study to Develop a Standardized Food Protein Solubility Procedure, *J. Food Sci. 50*:1715–1718 (1985).
11. McWatters, K.H., and M.R. Holmes, Influence of pH and Salt Concentration on Nitrogen Solubility and Emulsification Properties of Soy Flour, *Ibid. 44*:770–773, 781 (1979).
12. Pearce, K.N., and J.E. Kinsella, Emulsifying Properties of Proteins: Evaluation of Turbidimetric Technique, *J. Agric. Food Chem. 26*:716–723 (1978).
13. Sorgentini, D.A., J.R. Wagner, and M.C. Anon, Effects of Thermal Treatment of Soy Protein Isolate on the Characteristics and Structure–Function Relationship of Soluble and Insoluble Fractions, *Ibid. 43*:2471–2479 (1995).
14. Lin, C.S., and J.F. Zayas, Functionality of Defatted Corn Germ Proteins in a Model System: Fat Binding Capacity and Water Retention, *J. Food Sci. 52*:1308–1311 (1987).
15. Phillips, R.D., and M. Sternberg, Corn Protein Concentrate: Functional and Nutritional Properties, *Ibid. 44*:1152–1156 (1979).
16. Kinsella, J.E., Functional Properties of Proteins in Foods: A Survey, *Crit. Rev. Food Sci. Nutr. 7*:219–280 (1976).
17. Kinsella, J.E., Functional Properties of Soy Proteins, *J. Am. Oil Chem. Soc.* 56:242–256 (1979).
18. Zayas, J.F., *Functionality of Proteins in Food*, Springer, New York, 1997, p. 228.
19. Hutton, C.W., and A.M. Campbell, Functional Properties of Soy Concentrate and Soy Isolate in Simple Systems and in a Food System, *J. Food Sci. 42*:457–460 (1977).
20. Morr, C.V., Functionality of Oilseed and Legume Protein, *J. Am. Oil Chem. Soc. 67*:265–271 (1990).

[Received October 11, 2001; accepted August 2, 2002]