Functional Properties of Extruded-Expelled Soybean Flours from Value-Enhanced Soybeans

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: The functional properties (protein solubility, emulsification characteristics, foaming characteristics, water- and fat-binding capacities) of extruded-expelled (EE) soy flours originating from six varieties of value-enhanced soybeans (high-sucrose, high-cysteine, low-linolenic, low-saturated FA, high-oleic, and lipoxygenase-null) and two commodity soybeans were determined. The soy flours varied in protein dispersibility index (PDI) and residual oil (RO), with PDI values ranging from 32 to 50% and RO values ranging from 7.0 to 11.7%. Protein solubility was reduced at pH values near the isoelectric region and was higher at both low and high pH. There were no significant differences for water-holding capacity, fat-binding capacity, emulsification activity, or emulsification stability. Only the high-oleic soy flour had significantly lower emulsification capacity. In general, the PDI and RO values of EE soy flours originating from value-enhanced and commodity soybeans had the greatest influence on protein functionality. The genetic modifications largely did not affect functional properties.

Paper no. J10194 in *JAOCS 79,* 699–702 (July 2002).

KEY WORDS: Extrusion-expelling, functional properties, genetic modifications, low-fat soy flour, soy flour, soy protein, soybeans.

Soybeans are traditionally processed by solvent extraction using hexane. Today's solvent extraction plants require a large capital investment and the use of a hazardous and environmentally regulated solvent. One alternative to solvent extraction is the extruded-expelled (EE) method. This technology results in soybean meal containing approximately 6–11% oil depending on the processing conditions used (1). EE processing is gaining in popularity due to reduced capital investment, ease of running EE equipment, and the ability to process identity-preserved (IP) soybeans.

IP soybeans cannot be combined with any commodity-type soybean or any other IP soybean. Separation must be provided at every step of processing. In order to maintain IP, the equipment used for the transporting, processing, and storing of these soybeans must be thoroughly cleaned and inspected between uses. Documentation stating that the IP soybeans have been handled properly must follow each step of processing.

IP soybeans that offer marketing opportunities include soybeans that are organically produced, certifiable non-genetically modified, and specialty soybeans that are genetically enhanced for specific end-uses (i.e., soybeans with altered FA composition, increased sucrose content, altered protein composition). Although the acreage of IP soybeans is growing, the acreage is not now nearly as large as that of commodity-type soybeans. For this reason, many large soybean processors (processing over 1000 tons/day of soybeans) find it financially difficult, logistically challenging, and inefficient to process IP soybeans.

Functional properties have been defined by Kinsella (2) as "any physicochemical property which affects the processing and behavior of protein in food systems, as judged by the quality attributes of the final product." Functional properties or characteristics have been identified as being extremely important to examine before a new protein is used in a food or nonfood system. It should be noted that functional properties are performed in a model system, and the concrete function of the protein in the aforementioned applications remains unknown until it is incorporated into the intended food system. There are several potential interactions that the protein may face in a food system, for example, the interaction of protein with sugar and/or salt. However, performing protein functionality testing on the bench top is still a critical step to take before incorporating a protein in a food system.

There is little published work on protein functionality of value-enhanced soybeans. Furthermore, there is no available literature on utilizing EE-processed, value-enhanced soybeans and on the resulting functional properties of the protein. In the present study, EE processing was used to obtain low-fat soybean meal from six different value-enhanced soybean varieties. The value-enhanced varieties included high-sucrose (Hs: low in oligosaccharides), low-linolenic (LLL), lipoxygenase (Lox)-null, high-oleic (Ho), low-saturated fat (Ls), and high-cysteine (Hc). Hs soybeans have increased amounts of sucrose (6.7% sucrose compared with 5.7% for normal soybeans) and decreased amounts of stachyose (0.3% stachyose compared with 4.6% for normal soybeans). LLL soybeans have decreased amounts of the unsaturated FA linolenic acid (3.1% linolenic acid compared with 7.5% for normal soybeans). Lipoxygenase-null soybeans have all three lipoxygenase isozymes removed. Ho soybeans have increased amounts of the FA oleic acid (79.2% oleic acid compared with 25.2% for normal soybeans). Ls soybeans have decreased amounts of all saturated FA (8.4% total saturated FA compared with 15.7% for normal soybeans) (2). Hc soybeans have increased amounts of the amino acid cysteine in the 7S protein fraction. This increase is equivalent to five cysteine residues per mole of 7S protein. The objective of this study was to investigate the functional properties [including protein solubility, emulsification characteristics, foaming characteristics, and water-holding capacities (WHC) and fat-binding capacities (FBC)] of EE soy flours originating from different value-enhanced soybean varieties.

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

EXPERIMENTAL PROCEDURES

Soybean varieties. Table 1 shows the soybean varieties used in this study, the variety abbreviations, and the altered traits. In addition to the value-enhanced soybean varieties, two commodity soybeans were included as controls. Nongenetically-enhanced soybeans were obtained from two sources. Mixed bulk soybeans were from West Central Cooperative (Ralston, IA) and variety 3690-0 was from Stine Seed (Adel, IA). Optimum Quality Grains (Des Moines, IA) provided the Ho, Ls, Hs, and LLL soybeans. An experimental Hc line was obtained from the USDA, ARS at North Carolina State University (Raleigh, NC). Lox-null soybeans were provided by the Committee for Agricultural Development, Iowa State University (Ames, IA).

Processing of soy flour. Soybean processing took place at Iowa Soy Specialties (Vinton, IA) using EE equipment at their facility. Processing conditions were previously reported by Wang and Johnson (3). Low-fat soybean meal was ground into low-fat soy flour (100 mesh) using a pin mill (Bauermeister, Inc., Memphis, TN). Processing was replicated twice for each soybean variety.

Soy flour composition and protein dispersibility. Protein dispersibility index (PDI), as an indirect measure of protein solubility, was determined by an outside laboratory (Woodson-Tenant, Des Moines, IA) by using AOCS method Ba 10-65 (4). Residual oil (RO) was determined by using the Goldfisch extraction procedure (AACC 30-25) (5). Crude protein content was determined by using a nitrogen analyzer (PerkinElmer Corp., Norwalk, CT) and methods described by AOAC (4.2.08) (6).

Protein solubility. A sample (250 mg) was dispersed in 25 mL of distilled water and placed into a 50-mL centrifuge tube. The solution was adjusted to the appropriate pH with 1 N HCl or 1 N NaOH, shaken at 120 rpm at 25°C, and centrifuged at 30,500 × *g* for 30 min. This supernatant was then filtered through Whatman No.1 paper, and the nitrogen content was determined using 10 mL of the filtered supernatant and the standard Kjeldahl procedure (7). Protein solubility was calculated using the following equation:

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

Emulsification capacity (EC). The modified procedure of McWatters and Holmes (8) was used where a 2% protein suspension (25 mL) at 25°C was placed into a 500-mL beaker. The suspension was continuously blended with a handheld mixer at high speed (approximately 12,000 rpm) with soybean oil (Hy-Vee brand, West Des Moines, IA) at a flow rate of 0.5 g/s. This mixture was continuously blended until the inversion point (water-in-oil) was observed. Emulsification capacity was the maximum amount of oil emulsified per gram of protein.

Emulsification activity index (EAI) and emulsification stability index (ESI). A 2% protein suspension (25 mL) at 25°C was blended with 7 mL of soybean oil for 1 min using a Waring Blender equipped with a microcontainer (110-mL size; Fisher Scientific, Pittsburgh, PA) at low speed. The emulsion was immediately diluted with 0.1% SDS at a 500× dilution factor, and the absorbance measured at 500 nm. The diluted emulsion was then incubated at 95°C in a water bath, and the absorbance measured at time zero and at 10 min. The EAI and ESI were calculated using the equations of Pearce and Kinsella (9).

Foaming capacity (FC) and foam stability (FS). A 0.5% protein suspension (80 mL) at 25°C 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 FS were calculated using equations of Sorgentini *et al.* (10).

WHC. Modified methods of Lin and Zayas (11) were used to determine WHC. Low-fat soy flour (5 g) was dispersed into 95 mL of distilled water and mixed with a magnetic stirrer for 20 min at 25°C. Three 50-mL centrifuge tubes were filled with the flour-water solution and centrifuged at approximately 1,080 × *g* for 30 min. After the supernatant was discarded, the WHC was calculated as the difference in weight of the hydrated flour and the weight of the original flour. WHC was expressed as grams of water per gram of protein.

FBC. FBC was determined by stirring a 5% soy flour solution with 50 mL of corn oil (Hy-Vee brand) for 30 min followed by allowing this mixture to stand at room temperature for an additional 30 min (25°C). The mixture was then placed into two 50-mL centrifuge tubes and centrifuged for 30 min at approximately 1,080 × *g*. After the excess oil was disposed of, the FBC was calculated as the weight of the residue divided by the original weight (11). FBC was expressed as grams of oil per gram of protein.

Data analysis. All functionality testing followed a randomized complete block design. The General Linear Model procedure was used to determine treatment effects for all functionality tests. A Tukey test was used for multiple comparisons. Significance for all analyses was determined at the $P < 0.05$ level. Statistical evaluation was carried out using version 8.0 SAS statistical software (SAS Institute, Inc., Cary, NC, 1999).

TABLE 1
Soybean Varieties Used, Their Abbreviations, and Trait Modification

Soybean variety	Abbreviation	Trait altered[a]
Low-linolenic acid	LLL	Reduced unsaturated FA linolenic acid
High-sucrose	Hs	Reduced oligosaccharide, increased sucrose
Low-saturated fat	Ls	Reduced amounts of saturated FA
High-oleic acid	Ho	Increased FA oleic acid
High-cysteine	Hc	Increased amino acid cysteine
Lipoxygenase-null	Lox-null	Elimination of three lipoxygenase isozymes
Commodity	Wc	NA, bulk run at West Central Cooperative, Ralston, IA
Commodity	St	NA, var. 3690-0, Stine Seed, Adel, IA

[a]NA, not applicable.

RESULTS AND DISCUSSION

Soy flour composition and protein solubility. Table 2 shows the PDI and RO and protein contents of the soy flours. All soy flours were processed with the same configuration of the EE equipment and processing conditions. Thus, the variations in the PDI and RO levels were direct results of how the soybean variety performed in the EE process. RO was generally lower at lower PDI levels with the exception of Ho soybeans. This was due to greater exposure to heat and shear in the extruder and screw press, thus allowing a greater degree of cell disruption and pressure. Protein contents were relatively consistent, with the exception of Wc flour, which was significantly lower than the others.

Solubility. Solubility profiles for the low-fat soy flours are shown in Figure 1. Soy protein was least soluble at the protein's isoelectric region (pH 4.2–4.6) (12) and increased on either side of the isoelectric point. At pH 8.0, some of the soybean varieties showed greater solubility than did others. For example, Lox-null and St flours were more soluble than the LLL and Ls flours. This result was due to the PDI values of the aforementioned flours; Lox-null and St flours had PDI values of 50 and 49, respectively, whereas LLL and Ls flours had PDI values of 33 and 32, respectively.

Emulsification capacity, activity, and stability. All samples had similar EC values, except for the Ho flour. The EC for Ho flour was significantly lower than for Wc, St, and Hc flours (Fig. 2) although they had similar PDI values (Table 2). EC is greatly affected by PDI, protein conformation, and surface hydrophobicity (13). We speculate that the higher the RO, the greater the hydrophobicity of the sample. Although Ho flour had a similar PDI compared with the aforementioned low-fat soy flours, Ho flour had less RO and lower EC. In this instance, the effect of RO on the functionality of this protein exceeded that of PDI. There were no significant differences in EAI and ESI among any of the low-fat soy flours (Table 3).

FC and FS. FC was not affected by any of the value-enhanced traits, with the exception of the Hc flour (Table 4). We speculate that this difference was due to the increased amounts of intra- and intermolecular disulfide bonds, which stabilized the protein and did not allow for easy unfolding at the air/water interface during foam formation.

TABLE 2
Compositions and Protein Solubilities of Extruded-Expelled (EE)-Processed Soy Flours from Value-Enhanced and Commodity Soybeans

Soybean variety[a]	PDI (%)	RO (%)	Crude protein[b] (%)
LLL	32.2	7.7	52.1
Hs	35.5	7.0	52.4
Ls	32.0	7.1	51.8
Ho	45.2	7.5	51.8
Hc	42.7	9.0	51.2
Lox-null	49.5	11.7	52.6
Wc	41.2	11.0	47.4
St	48.7	10.5	50.1

[a]Soybean varieties: LLL, low-linolenic; Hs, high-sucrose; Ls, low-saturated fat; Ho, high-oleic; Hc, high-cysteine; Lox-null, lipoxygenase-null; Wc and St, commodity soybeans.
[b]Dry moisture basis.

TABLE 3
Emulsification Activity (EAI) and Stability Indices (ESI) of EE-Processed Soy Flours from Value-Enhanced and Commodity Soybeans[a]

Soybean variety	EAI (m^2/g protein)	ESI (min)
LLL	17.5	22.1
Hs	15.7	22.7
Ls	16.6	18.8
Ho	18.0	23.9
Hc	15.6	19.0
Lox-null	14.0	18.9
Wc	15.7	19.7
St	14.4	18.3
	NS	NS

[a]NS indicates all values in the column are not significantly different at $P < 0.05$. See Table 2 for other abbreviations.

The lower the FS value, the more stable the foam. All other flours had less stable foams than did Hc flours (significantly different for LLL), yet they had higher FC. This result indicates that the Hc flour is unable to form large amounts of foam; however, the foam that is produced is stable to leakage and breakdown. The difference is that at the air/water interface of foams, proteins undergo much more rigorous denaturation. For this reason, given opportunity, protein from Hc flour denatures and reconforms with the capacity to form strong, flexible films.

FBC and WHC. There were no significant differences in FBC among the flours of the different soybean varieties (Table 5). There was a slight increase in WHC for the Hc flour, which we attributed to the increased content of cysteine, a polar amino acid capable of cross-linking. FBC was not related to RO. Earlier work on low-fat soy flour speculated that increased RO would increase FBC and decrease WHC (15), but our results did not confirm this.

ACKNOWLEDGMENTS

Partial funding for this research was provided by the Iowa Soybean Association (West Des Moines, IA), the Iowa Agriculture and Home Economics Experiment Station, and the Center for Crops Utilization Research. The authors would like to acknowledge Troy Crowe and Dr. Tong Wang for their assistance in producing the soy flours and Iowa Soy Specialties for allowing the use of their EE equipment.

TABLE 4
Foaming Capacities (FC) and Stabilities (FS) of EE-Processed Soy Flours from Value-Enhanced and Commodity Soybeans[a]

Soybean variety	FC (mL of foam/mL of N_2 × min)	FS (mL^{-1} min^{-1})
LLL	2.15	1.04^{b}
Hs	2.57	$0.71^{a,b}$
Ls	2.39	$0.70^{a,b}$
Ho	2.25	$0.75^{a,b}$
Hc	1.98	0.24^{a}
Lox-null	2.20	$0.45^{a,b}$
Wc	2.39	$0.41^{a,b}$
St	2.26	$0.39^{a,b}$
	NS	

[a]Means with different roman superscripts are significantly different at $P < 0.05$. NS indicates all values in column are not significantly different. See Tables 2 and 3 for other abbreviations.

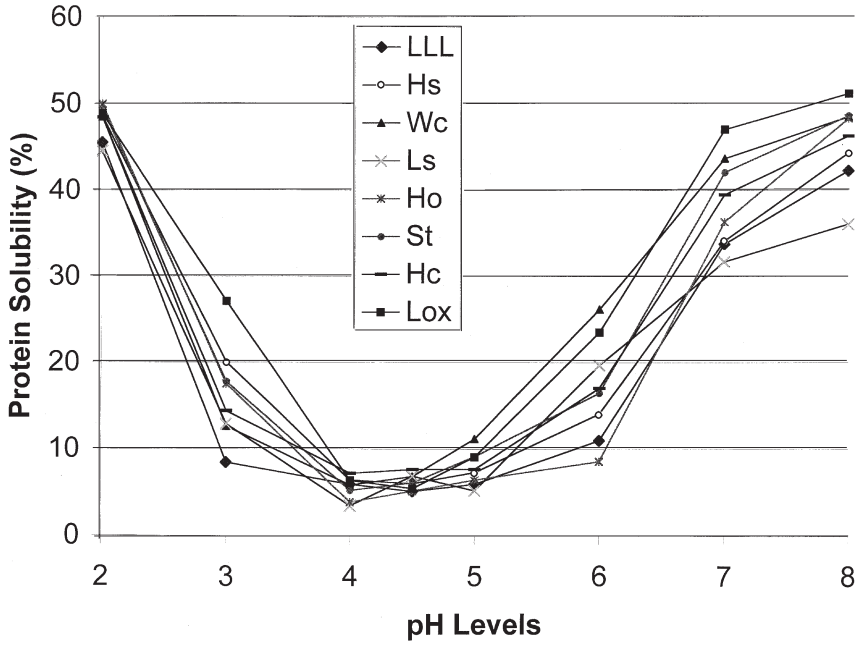

FIG. 1. Protein solubility curves of extruded-expelled value-enhanced and commodity soy flours. Soybean varieties: LLL, low-linolenic; Hs, high-sucrose; Ls, low-saturated-fat; Ho, high-oleic; Hc, high-cysteine; Lox-null, lipoxygenase-null; and Wc and St, commodity soybeans.

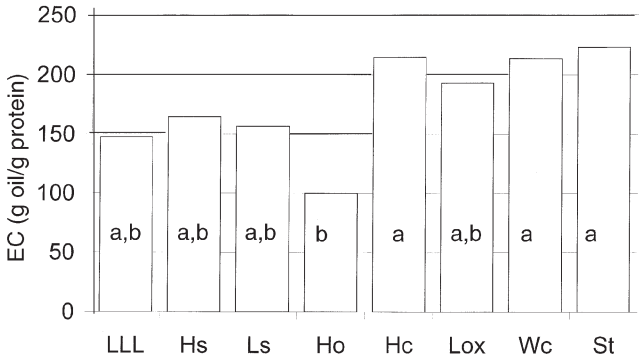

FIG. 2. Emulsification capacities (EC) for EE-processed value-enhanced and commodity soy flours. Soybean varieties with the same letter on bars are not significantly different at $P < 0.05$. See Figure 1 for other abbreviations.

TABLE 5
Fat-Binding Capacities (FBC) and Water-Holding Capacities (WHC) of EE-Processed Soy Flours from Value-Enhanced and Commodity Soybeans

Soybean variety	FBC (g oil/g protein)	WHC (g water/g protein)
LLL	2.1	3.9
Hs	2.0	3.8
Ls	2.2	3.7
Ho	2.2	3.9
Hc	2.0	4.2
Lox-null	2.0	3.7
Wc	2.1	4.1
St	1.9	3.7
	NS	NS

[a]NS indicates all values in the column are not significantly different at $P < 0.05$. See Table 2 for other abbreviations.

REFERENCES

1. Said, N.W., Quality Control of Extruded Soybeans and Extruded/Expelled Soy Meal, Ref. 69.1015, Insta-Pro International, Des Moines, IA (1998).
2. Kinsella, J.E., Structure and Functional Properties of Food Proteins, in *Food Proteins*, edited by P.F. Fox and J.J. Condon, Applied Science Publishers, New York, 1982, pp. 51–103.
3. Wang, T., and L.A. Johnson, Refining Normal and Genetically Enhanced Soybean Oils Obtained by Various Extraction Methods, *J. Am. Oil Chem. Soc. 78*:809–815 (2001).
4. AOCS, *Official Methods of the American Oil Chemists' Society*, 4th edn., American Oil Chemists' Society, Champaign (1993).
5. AACC, *American Association of Cereal Chemists Approved Methods,* 8th edn., American Association of Cereal Chemists, St. Paul (1983).
6. AOAC, *Official Methods of Analysis,* 16th edn., Association of Official Analytical Chemists, Washington, DC (1995).
7. 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).
8. 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).
9. Pearce, K.N., and J.E. Kinsella, Emulsifying Properties of Proteins: Evaluation of Turbidimetric Technique, *J. Agric. Food Chem. 26*:716–723 (1978).
10. 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).
11. 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).
12. Kinsella, J.E., Functional Properties of Soy Proteins, *J. Am. Oil Chem. Soc. 56*:242–258 (1979).
13. Heywood, A.A., Characterization and Utilization of Extruded-Expelled Soy Flours, Iowa State University Thesis, Ames, IA (2001).

[Received December 19, 2001; accepted April 6, 2002]