Functional Properties of Protein Ingredients Prepared from High-sucrose/Low-stachyose Soybeans

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ABSTRACT: High-sucrose/low-stachyose (HS/LS) soybeans were used to prepare ethanol-washed soy protein concentrate (EWSPC), soy protein isolate (SPI), and a new low-fiber soy protein concentrate (LFSPC) in which the protein was extracted with alkali to remove fiber and the protein extract was neutralized and freeze-dried. LFSPC prepared from HS/LS soybeans contained significantly higher ratios of β-conglycinin to glycinin (1:1.32) than did EWSPC (1:1.75) or SPI (1:1.69), which may have affected functional properties. The LFSPC were also high in soluble sugars (14.7%) and low in fiber (0.3%) compared with traditional EWSPC (2.9 and 3.4%, respectively) and SPI (1.8 and 0.3%, respectively). For both normal and HS/LS soybean varieties, the LFSPC, especially when extracted at pH 7.5 as opposed to pH 8.5, had higher denaturation enthalpies than did EWSPC and SPI, indicating less denaturation had occurred. Water solubilities, surface hydrophobicities, and emulsification properties were highest for the LFSPC and lowest for EWSPC. The LFSPC also had good foaming properties and low viscosities. These desirable functional properties of the LFSPC make them unique among alternative soy protein ingredients and highly suitable for industrial applications as food additives and ingredients.

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KEY WORDS: High-sucrose soybeans, low-stachyose soybeans, protein, protein functionality, soybeans, soy protein, soy protein concentrate, soy protein isolate.

Consumer demand for soy foods and soy-protein food ingredients is rapidly increasing. U.S. retail sales of foods containing soy protein have grown by more than 10% per year for the last seven years, reaching an estimated annual retail market of $3.65 billion in 2002 (1). The current driving force regarding soy in the food industry is increased recognition of the health properties of soy protein. This growth in consumer demand, however, is limited by poor flavor, presence of antinutritional factors, flatus-causing sugars, and limited functionality (2). Producing new products with enhanced health benefits and superior functional properties is key to further increasing consumption of soy products.

There are a number of ways in which soybean breeding can improve soybeans for use as food. Reducing the contents of indigestible and flatus-causing sugars is one example (1), and this improvement was recently achieved by Pioneer Hi-Bred International, Inc., a DuPont Company (Johnston, IA). In previous work (3), we reported on the compositional characteristics of new low-fiber soy protein concentrates (LFSPC) based on a new soybean variety that was bred to contain high sucrose and low stachyose levels (3). We also used a different processing approach in which defatted soy flour was extracted with alkali, neutralized, and dried (4,5). Defatted soy flour prepared from high-sucrose/low-stachyose (HS/LS) beans typically contains ~0.7% stachyose and ~10.5% sucrose (3) compared with ~4.7 and ~5.7%, respectively, for defatted soy flour prepared from normal soybeans (6). Even though the soluble sugars are present in LFSPC, the modified sugar contents do not need to be removed because they are largely digestible, do not cause flatulence, and contribute sweetness. Except for full-fat and defatted soy flour, soluble sugars are removed when preparing all other soy protein ingredients such as soy protein concentrate and soy protein isolate (SPI). LFSPC prepared from HS/LS soybeans contained significantly higher ratios of β-conglycinin to glycinin than did ethanol-washed soy protein concentrates (EWSPC) or SPI that may affect functional properties (3). The LFSPC were also high in soluble sugars and low in fiber compared with traditional EWSPC and SPI.

Although most of the soy protein in the United States is used as toasted meal for feeding livestock, a growing proportion of this inexpensive protein is used to produce refined food ingredients (2). The utilization of soy protein as a food ingredient is largely based on useful functional properties such as thermal behavior, solubility, foaming, emulsification, and viscosity control. These functional properties are the physicochemical characteristics of proteins that determine their behavior and performance in food systems during processing, storage, food preparation, and consumption (7). The desired functional properties, and as a consequence the applications for which they are useful, vary from product to product (8). Soy flours, soy protein concentrates (SPC), and SPI have distinctly different applications in food products.

Several factors influence the functional properties of protein ingredients, including intrinsic, environmental, and processing (7). The development of any new soy protein ingredient requires functional characterization to identify food applications where it has competitive advantages. The functional properties will determine its value and application in different food systems. In the present work, our central hypothesis was that the differences in the composition of HS/LS soybeans and the method of producing a new SPC result in functional properties

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that are different from those of traditional SPC and SPI. The objectives of this study were to characterize the functional behavior of LFSPC, compare the results obtained using two different extraction pH values, and compare the functional properties of LFSPC to traditional EWSPC and isoelectric-precipitated SPI.

EXPERIMENTAL PROCEDURES

Preparation of soy flours and protein ingredients. All protein ingredients were prepared from air-desolventized, hexane-defatted white flakes from control normal soybeans (IA2020 variety, 1999 harvest) and HS/LS white flakes (2 HS Soybeans, Low Stachyose, Lot-980B0001 OPTIMUM, 1999 harvest; Pioneer a DuPont Company, Johnston, IA). Both white flake samples were produced from soybeans extracted in the pilot plant of the Center for Crops Utilization Research by using a French Oil Mill Machinery Co. extractor-simulator (Piqua, OH). Triplicate runs from each flour type and for each of the four procedures [LFSPC prepared at two different extraction pH values (8.5 and 7.5), EWSPC, and SPI] were prepared according to procedures described in our previous work (8). The freeze-dried products were stored in sealed containers at 4°C until used.

Thermal behavior. Thermal behaviors of the protein products were determined by using DSC. Samples (15–20 mg) of 10% (w/w, dry basis) dispersions were hermetically sealed in aluminum pans. Sealed, empty pans were used as references. The samples were heated from 25 to 120°C at 10°C/min by using an SII Exstar 6000 DSC (Seiko Instrument, Inc., Tokyo, Japan). All samples were analyzed at least three times and means reported.

Solubility. Solubility was evaluated according to methods of Rickert *et al.* (8) by preparing 1% (w/w dry basis) sample dispersions in de-ionized water. The pH was adjusted over the range 2.0 to 11.0 by using 2 N HCl or NaOH, the volume of acid or base recorded, and initial protein content calculated based on the change in volume. The dispersions were stirred for 1.0 h. Aliquots (25 mL) of the dispersions were transferred to 50-mL centrifuge tubes and centrifuged at 10,000 × *g* and 20°C for 10 min. The protein content of the supernatant was measured by using the Biuret method with BSA (Sigma Chemical, St. Louis, MO) as the reference standard. Solubility was calculated as: % solubility = (amount of protein in the supernatant/initial amount of protein) × 100.

Surface hydrophobicity. Surface hydrophobicity was measured by using the methods of Wu *et al*. (9) with 1-anilino-8-naphthalene sulfonic acid magnesium salt monohydrate (ANS; ICN Biomedicals, Inc., Aurora, OH). Protein dispersions (prepared as in the solubility test) were stirred, adjusted to pH 7.0, and centrifuged at 10,000 × g and 20°C for 10 min. Aliquots of soluble protein (supernatant) were serially diluted with 0.1 M phosphate buffer (pH 7.0) to obtain 6.25 to 100 µg/mL protein. Forty microliters of ANS (8.0 mM in 0.01 M phosphate buffer, pH 7.0) was dispersed in 3-mL aliquots of each protein dilution. Fluorescence intensity units (FIU) were measured by using a Turner Quantech spectrophotometer (Barnstead Thermolyne, Dubuque, IA) with 440-nm (excitation) and 535-nm (emission) filters. FIU were standardized by using a solution of 40 µL ANS in 3 mL of phosphate buffer as the zero point; 15 µL ANS in 3 mL of methanol was assigned an arbitrary value of 80 FIU. FIU were plotted against protein concentration. The slope of the regression line was reported as surface hydrophobicity. Samples were run in triplicate and means reported.

Emulsification properties. Emulsification capacity (EC) was measured according to the method of Bian *et al*. (10) with modifications. Dispersions (25 mL) of 2% (w/w, dry basis) sample were adjusted to pH 3.0, 4.0, 5.0, or 7.0 with 2 N HCl or NaOH as needed, the volume of acid or base was recorded, initial solids were adjusted based on the changes in volume, and solutions were transferred to 400-mL plastic beakers. Soybean oil, dyed with approximately 4 ppm Sudan Red 7B (Sigma Chemical), was continuously blended into the protein dispersions at 37 mL/min flow rate by using a Bamix wand mixer (ESGE AG Model 120; Mettlen, Switzerland) at the low setting until phase inversion was observed. EC (g oil/g sample) was calculated with the following formula: EC (g oil/g sample) = (g of oil needed to cause inversion/25 mL 2% sample dispersion)*2. Samples were run in at least triplicate and means reported.

Emulsification activity (EA) and emulsification stability index (ESI) were measured according to methods of Rickert *et al*. (8). Dispersions (21 mL) of 2% (w/w, dry basis) samples were adjusted to pH 3.0, 4.0, 5.0, or 7.0 with 2 NHCl or 2 N NaOH as needed, the volume of acid or base was recorded, initial solids were adjusted based on the changes in volume, and the resultant mixtures were blended with 7 mL refined soybean oil (Bakers' and Chefs' Vegetable Oil; North Arkansas Wholesale Company Inc., Bentonville, AR) in a 250-mL glass beaker for 1.0 min by using the Bamix wand mixer at low speed. Immediately after mixing, the emulsion was diluted 1:1000 with 0.1% SDS. The absorbance was measured at 500 nm and recorded as EA. After 15 min, the absorbance was measured again. These two absorbance readings were used to calculate ESI as: ESI (min) = $[A_0/(A_0 - A_{15})]t$ where A_0 and A_{15} were the absorbance at time 0 and 15 min, respectively, and t was the time interval. Samples were run in triplicate and means reported.

Foaming properties. Foaming capacity (FC), foaming stability (K), and rate of foaming (V_i) were measured according to methods of Sorgentini *et al*. (11) with modifications (8). A 0.5% (w/w, dry basis) sample dispersion was prepared and the pH adjusted to 7.0. A 95-mL aliquot was loaded into a custom-designed glass column (58.5 × 2 cm) fitted with a coarse glass frit at the bottom, and nitrogen gas was purged through the sample at 100 mL/min flow rate. The time for the foam to reach the 300-mL mark, the time for one-half of the liquid incorporated into the foam to drain back, and the volume of the liquid incorporated into the foam were measured. Three parameters were calculated: (i) FC = $V_f/(f_r \times t_f)$; (ii) K (specific rate constant of drainage) = $1/(V_{max} \times t_{1/2})$; and (iii) V_i (rate of liquid conversion to foam) = V_{max}/t_f, where V_f = the fixed volume of 300 mL, f_r = the flow rate of the gas, t_f = time to reach V_f, V_{max}

= the volume of liquid incorporated into foam, and $t_{1/2}$ = the time to drain one-half of the liquid incorporated into the foam. Samples were run in triplicate and means reported.

Dynamic viscosity. A 10% (w/w, dry basis) sample dispersion was prepared at pH 7.0 (8). The sample was applied to the plate of a RS-150 Rheo Stress rheometer (Haake, Karlsruhe, Germany), and shear was applied with a 60-mm 2° titanium cone (C60/2 Ti) from 10 to 500/s shear rate, at constant temperature (23°C). Shear rate (γ) and shear stress (τ) over the course of the analysis, in combination with the power-law formula application, were used to determine the consistency coefficient (k) and flow behavior index (n), where $\tau = k\gamma^n$. Using k, n, and γ, apparent viscosity (η) was estimated by using the equation $\eta = k\gamma^{n-1}$. Samples were run in triplicate and means reported.

Statistical analyses. The data were analyzed by using ANOVA and the General Linear Model, and least significant differences (LSD) were calculated at the 5% level to compare treatment means using the SAS system (version 8.2; SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Thermal behavior. Thermal behavior of protein, especially denaturation enthalpy, is an indicator of the extent of denaturation that the protein has undergone prior to analysis. Energy is required to disrupt protein conformation and cause unfolding. The denaturation shifted to significantly lower temperatures for EWSPC and SPI, probably caused by partial denaturation of the proteins by either ethanol or acid that reduced the activation energy required for denaturation (12).

The LFSPC extracted at pH 8.5 had lower denaturation enthalpies for both the glycinin and β-conglycinin components than the LFSPC extracted at pH 7.5 (Table 1). The peak denaturation temperature for β-conglycinin was slightly lower for the LFSPC extracted from HS/LS soy flour at pH 8.5 than for the LFSPC extracted HS/LS soy flour at pH 7.5. These decreased values were attributed to greater denaturation during alkali extraction at the higher pH.

The β-conglycinin components of LFSPC prepared from HS/LS soybeans were less thermally active than the same products made from IA2020 soybeans and for both extraction pH values. Interestingly, the LFSPC made from HS/LS soybeans had significantly more β-conglycinin (3) than the same ingredients made from IA2020 soybeans, but this component was more readily denatured. The thermal behaviors of the glycinin components were similar for both varieties.

When comparing the LFSPC with the traditional soy protein ingredients, the LFSPC extracted at pH 8.5 had denaturation enthalpies similar to those of SPI and significantly higher than those of EWSPC. The LFSPC extracted at pH 7.5, however, had significantly higher denaturation enthalpies than SPI and EWSPC. For both soybean types, EWSPC had substantial thermal activities, probably because they were air desolventized. In general, the protein products prepared from HS/LS soybeans had lower denaturation enthalpies for β-conglycinin and higher enthalpies for glycinin, when comparing the protein products from the two soybeans. One possible explanation for this phenomenon is that the subunit makeup of the β-conglycinin component was different between the two soybean varieties and affected the thermal behavior of this protein (13).

TABLE 1
Thermal Properties of Protein Ingredients Prepared from Normal and High-sucrose/Low-stachyose Soybeans[a]

Soybeans/ protein product	Denaturation peak temperature (°C)		Enthalpy (mJ/mg of protein)	
	β-Conglycinin	Glycinin	β-Conglycinin	Glycinin
IA2020 Soybeans				
Defatted flour				
LFSPC, pH 7.5	74.2[a]	94.4[a]	2.17[a]	8.35[a]
LFSPC, pH 8.5	73.9[a,b]	93.8[a]	1.14[c]	7.27[c]
SPI	73.4[b,c]	91.9[b]	1.18[b,c]	7.25[c]
EWSPC	71.7[d]	89.7[c]	0.83[d]	6.89[d]
HS/LS soybeans				
Defatted flour				
LFSPC, pH 7.5	73.9[a,b]	94.0[a]	1.40[b]	8.66[a]
LFSPC, pH 8.5	72.9[c]	94.4[a]	0.78[d]	7.71[b]
SPI	72.9[c]	92.5[b]	0.67[d]	7.77[b]
EWSPC	71.3[d]	89.1[c]	0.64[d]	6.41[e]
LSD	0.6	0.7	0.22	0.33

[a]$n = 3$. Means within a column followed by different superscripts are significantly different at $P < 0.05$. HS/LS denotes high-sucrose/low-stachyose soybeans; IA2020, a line of normal soybeans; LFSPC, low-fiber soy protein concentrate prepared by alkali extracting and then neutralizing and drying; pH 7.5 and 8.5, extraction pH for LFSPC; SPI, soy protein isolate; EWSPC, ethanol-washed soy protein concentrate; and LSD, least significant difference.

TABLE 2
Protein Solubilities of Soy Protein Ingredients Prepared from Normal and HS/LS Soybeans(%)[a]

Soybeans/ protein product	pH 2	3	4	5	6	7	8	9	10	11
IA2020 soybeans										
LFSPC, pH 7.5	96.9^{a}	94.8^{a,b}	21.8^{a}	19.8^{a}	91.8^{a}	98.2^{a}	99.3^{a}	98.8^{a}	98.9^{a}	99.2^{a}
LFSPC, pH 8.5	98.6^{a}	96.6^{a}	20.3^{a}	17.8^{b}	92.9^{a}	98.7^{a}	98.7^{a}	97.7^{a}	98.5^{a}	98.2^{a}
SPI	89.9^{a}	87.9^{c}	24.4^{a}	7.3^{d}	87.0^{b}	91.0^{b}	90.7^{b}	90.2^{b}	90.7^{b}	90.3^{b,c}
EWSPC	41.0^{f}	16.3^{e}	4.3^{c}	4.8^{e}	15.0^{d}	20.3^{d}	26.6^{d}	34.8^{c}	44.5^{d}	78.9^{d}
HS/LS soybeans										
LFSPC, pH 7.5	85.2^{d}	84.5^{d}	10.9^{b}	7.6^{d}	66.0^{c}	85.9^{c}	86.1^{c}	88.5^{b}	87.3^{c}	88.1^{c}
LFSPC, pH 8.5	96.7^{a}	92.3^{b}	18.7^{a}	14.1^{c}	84.9^{b}	96.8^{a}	99.6^{a}	100.7^{a}	100.7^{a}	100.4^{a}
SPI	87.6^{c}	87.1^{c,d}	12.0^{b}	0.9^{f}	85.7^{b}	88.3^{c}	88.6^{b,c}	89.9^{b}	91.0^{b}	90.7^{b}
EWSPC	44.4^{e}	15.1^{e}	6.0^{b,c}	4.8^{e}	8.7^{e}	12.7^{e}	18.4^{e}	22.3^{d}	39.1^{e}	75.2^{e}
LSD	2.1	3.0	6.1	1.5	2.9	2.5	3.5	3.4	3.4	2.3

[a]$n = 3$. Means within a column followed by different superscripts are significantly different at $P < 0.05$. For abbreviations see Table 1.

Solubility. The protein solubilities over the pH range 2–11 for all protein fractions are shown in Table 2. Both LCSPC were highly soluble and exhibited the characteristic U-shaped solubility curves when solubility was plotted against pH. The LCSPC had significantly higher solubilities than either EWSPC and similar solubilities to those of SPI. The solubility of EWSPC was quite poor unless it was jet-cooked or alkaline-homogenized to break up denatured protein aggregates (14). The solubilities in the high (7–11) and the low (2–3) pH ranges for the LFSPC extracted at pH 8.5 were significantly higher than the solubilities for SPI, whereas the LFSPC extracted at pH 7.5 was less soluble.

Both LCSPC and SPI prepared from IA2020 soybeans had significantly ($P < 0.05$) higher solubilities than the same products produced from HS/LS soybeans. It seemed that the protein in HS/LS soybeans was more readily solubilized from the soy flour matrix (3), especially the β-conglycinin component, but at the same time this protein was more readily denatured, aggregated, and lost solubility.

Surface hydrophobicity. Physicochemical properties of proteins at interfacial surfaces affect film formation, dispersibility and solubility, emulsification, interaction with other ingredients, and other properties important in foods. Surface hydrophobicity depends on two main factors: the combination of denaturation processes (heat, alkali, acid, ethanol, etc.), which tend to increase surface hydrophobicity by unfolding the protein structure and exposing hydrophobic regions, and the aggregation phenomena that tend to decrease surface hydrophobicity by means of protein–protein interactions and consequential reduction of exposed hydrophobic regions (12).

The LCSPC had significantly higher surface hydrophobicities than EWSPC. In general, the EWSPC had very low solubilities and surface hydrophobicities, probably owing to the formation of large protein aggregates during ethanol extraction. In addition, the ANS probe only measures the hydrophobicity of soluble proteins, making data interpretation for EWSPC difficult. The LFSPC extracted at pH 8.5 and the SPI had high surface hydrophobicities, and the LFSPC extracted at pH 7.5 had lower surface hydrophobicities. Apparently, the acid treatment used in precipitation did not significantly affect the surface hydrophobicity of SPI, but the higher extraction pH (common to both the LFSPC pH 8.5 and SPI procedures) did affect surface hydrophobicity. This latter observation is in good agreement with data reported by Petrucelli and Añón (12).

For both normal soybeans and HS/LS soybeans, the LCSPC extracted at pH 8.5 had significantly higher surface hydrophobicities than the LCSPC extracted at pH 7.5 (Table 3). We believe this effect was due to the greater extent of protein denaturation of the LFSPC extracted at pH 8.5. The loss of the native state causes unfolding of globular proteins with the consequential exposure of hydrophobic regions and increased surface hydrophobicity. In addition, the LCSPC extracted at pH 8.5 had higher ash (salt) contents than the LCSPC extracted at pH 7.5 (3). The higher ash contents may have led to higher surface hydrophobicities of these protein products, since the ANS probe is known to be salt-sensitive (15).

TABLE 3
Surface Hydrophobicities (dimensionless) of Protein Ingredients Prepared from Normal and HS/LS Soybeans at pH 7.0[a]

Soybeans/ protein product	Surface hydrophobicity (dimensionless)
IA2020 soybeans	
LFSPC, pH 7.5	267^{d}
LFSPC, pH 8.5	379^{a}
SPI	351^{b}
EWSPC	56^{e}
HS/LS soybeans	
LFSPC, pH 7.5	297^{c}
LFSPC, pH 8.5	353^{b}
SPI	383^{a}
EWSPC	63^{e}
LSD	13

[a]$n = 3$. Means followed by different superscripts are significantly different at $P < 0.05$. For abbreviations see Table 1.

TABLE 4
Emulsification Properties of Protein Ingredients Prepared from Normal and HS/LS Soybeans at Different pH Values[a]

Emulsification property/soybeans/ protein product	pH 3	pH 4	pH 5	pH 7
Emulsification capacity (g of oil emulsified/g of product)				
IA2020 soybeans				
LFSPC, pH 7.5	437^{c}	314^{c}	338^{d}	486^{d}
LFSPC, pH 8.5	447^{c}	343^{b}	392^{a}	527^{c}
SPI	541^{a}	341^{b}	360^{b,c}	583^{b}
EWSPC	223^{d}	125^{d}	95^{e}	334^{f}
HS/LS soybeans				
LFSPC, pH 7.5	460^{b,c}	376^{a}	348^{c,d}	501^{d}
LFSPC, pH 8.5	474^{b}	350^{b}	341^{c,d}	538^{c}
SPI	524^{a}	315^{c}	369^{b}	617^{a}
EWSPC	209^{d}	125^{d}	93^{e}	360^{e}
LSD	26	16	20	20
Emulsification activity (absorbance at 500 nm)				
IA2020 soybeans				
LFSPC, pH 7.5	0.160^{c}	0.075^{e}	0.074^{c}	0.233^{b}
LFSPC, pH 8.5	0.216^{a}	0.093^{d}	0.096^{a,b}	0.245^{b}
SPI	0.179^{b}	0.138^{a}	0.073^{c}	0.283^{a}
EWSPC	0.060^{d}	0.025^{f}	0.031^{d}	0.089^{c}
HS/LS soybeans				
LFSPC, pH 7.5	0.150^{c}	0.106^{c}	0.082^{b,c}	0.226^{b}
LFSPC, pH 8.5	0.216^{a}	0.096^{d}	0.101^{a}	0.246^{b}
SPI	0.160^{c}	0.125^{b}	0.072^{c}	0.280^{a}
EWSPC	0.031^{e}	0.020^{f}	0.020^{d}	0.078^{c}
LSD	0.017	0.010	0.015	0.027
Emulsification stability index (dimensionless)				
IA2020 soybeans				
LFSPC, pH 7.5	62^{d}	28^{c}	25^{f}	76^{f}
LFSPC, pH 8.5	83^{c}	26^{c}	26^{e,f}	97^{e}
SPI	107^{b}	38^{a}	46^{a}	175^{b}
EWSPC	33^{e}	25^{c}	28^{d,e,f}	41^{g}
HS/LS soybeans				
LFSPC, pH 7.5	71^{d}	36^{b}	31^{c,d,e}	129^{d}
LFSPC, pH 8.5	119^{a}	34^{b}	38^{b}	157^{c}
SPI	125^{a}	45^{a}	35^{b,c}	191^{a}
EWSPC	40^{e}	49^{a}	33^{b,c,d}	48^{g}
LSD	9	5	5	10

[a]$n = 3$. Means within a column followed by different superscripts are significantly different at $P < 0.05$. For abbreviations see Table 1.

Emulsification properties. The emulsification properties of a protein depend on two factors: the ability to reduce interfacial tension because of the adsorption of the protein to the interface and the ability to form a film so that the protein acts as an electrostatic, structural, and mechanical barrier. To achieve good emulsification properties, protein molecules must have both hydrophilic and hydrophobic regions and maintain flexibility in order to unfold. Emulsions are thermodynamically unstable, and once formed, an emulsion can undergo a number of changes. It is of interest to know not only how efficient a protein dispersion is in creating an emulsion but also how stable it is. Creating an emulsion depends on rapid adsorption, unfolding at the interface, and reorientation, whereas stability is determined by the decrease in interfacial free energy and the rheological properties of the film (16).

EC, EA, and ESI data on a dry-weight basis are shown in Table 4. The LCSPC had emulsification properties superior to those of EWSPC, which was consistent with both solubility and surface hydrophobicity data. EWSPC have very low emulsification capacities (14). The LCSPC had emulsification properties similar to those of SPI on a dry-weight basis. When the emulsification properties were expressed on a protein basis (by converting data in Table 4), the LCSPC had emulsification properties superior to SPI because the LCSPC contained less protein than SPI (65 vs. 90%).

TABLE 5
Foaming Properties of Protein Ingredients Prepared from Normal and HS/LS Soybeans[a]

Soybeans/ protein product	Foaming capacity (mL/mL)	Foaming stability $(mL \cdot min)^{-1}$	Rate of foaming (mL/min)
IA2020 soybeans			
LFSPC, pH 7.5	1.425[a]	0.0104[d,e,f]	21.8[a]
LFSPC, pH 8.5	1.258[c]	0.0122[d,e]	16.1[b]
SPI	1.096[d]	0.0133[c,d]	11.1[c]
EWSPC	0.949[e]	0.0216[b]	3.8[e]
HS/LS soybeans			
LFSPC, pH 7.5	1.360[a,b]	0.0092[e,f]	22.5[a]
LFSPC, pH 8.5	1.299[b,c]	0.0086[f]	20.7[a]
SPI	1.377[a,b]	0.0157[c]	6.5[d]
EWSPC	0.963[e]	0.0735[a]	5.1[d,e]
LSD	0.090	0.0031	2.3

[a]$n = 3$. Means within a column followed by different superscripts are significantly different at $P < 0.05$. For abbreviations see Table 1.

On a protein basis, the EC of the LFSPC extracted at pH 8.5 were 715, 528, 514, and 811 g of oil emulsified/g of protein at pH 3, 4, 5, and 7, respectively. The LFSPC extracted at pH 7.5 had EC of 691, 565, 523, and 753 g of oil emulsified/g of protein at pH 3, 4, 5, and 7, respectively. The SPI prepared from the IA2020 soybeans had EC of 593, 374, 394, and 638 g of oil emulsified/g of protein at pH 3, 4, 5, and 7, respectively. Similarly, after converting EA data to a protein basis, the LFSPC extracted at pH 8.5 had EC of 0.326, 0.145, 0.152, and 0.371 g of oil emulsified/g of protein; the LFSPC extracted at pH 7.5 had EC of 0.225, 0.159, 0.123, and 0.339 g of oil emulsified/g of protein; whereas the SPI had EC of 0.196, 0.151, 0.080, and 0.310 g of oil emulsified/g of protein, for pH 3, 4, 5, and 7, respectively. On a protein basis, the ESI for the LFSPC extracted at pH 8.5 were 179, 51, 57, and 237 at pH 3, 4, 5, and 7, respectively; the ESI for the LFSPC extracted at pH 7.5 were 107, 54, 47, and 194; and the ESI for SPI were 117, 42, 50, and 192 for pH 3, 4, 5, and 7, respectively. The additional exposure of the protein in SPI to acid during precipitation may account for the poorer emulsification properties for SPI than LFSPC on an equivalent protein basis. This was probably due to denaturation/aggregation of soy proteins during SPI production and as a consequence a loss of flexibility in the protein molecules.

The LFSPC extracted at pH 8.5 had significantly higher EC at pH 7 and significantly lower EC at pH 4 than the LFSPC extracted at pH 7.5. Both LCSPC had similar EC at pH 3 and 5. The LFSPC extracted at pH 8.5 had higher EA and ESI at pH 4, 5, and 7 compared with the LFSPC extracted at pH 7.5. Both LCSPC had poor emulsification properties at pH 4 and 5 owing to the close proximity to the isoelectric point for soy protein. The superior emulsification properties of the LFSPC extracted at pH 8.5 compared with the LFSPC extracted at pH 7.5 were attributed to their higher solubility and surface hydrophobicity.

The LCSPC prepared from HS/LS soybeans had similar EC over the pH range tested and superior ESI to LCSPC prepared from IA2020 soybeans. This observation was not expected based on our data on thermal behavior, solubility, and surface hydrophobicity data. The LCSPC prepared from HS/LS soybeans emulsified as much oil as did the LCSPC prepared from IA2020 soybeans but were more effective in stabilizing these emulsions. The improved emulsion stabilization of LCSPC prepared from HS/LS soybeans was probably due to the higher proportions of β-conglycinin present in the protein portion than the LCSPC prepared from IA2020 soybeans. β-Conglycinin is reported to have emulsification properties superior to those of glycinin (8,10).

Foaming properties. The amphipathic character of the side chains of the amino acids comprised in soy protein is responsible for their adsorption at interfaces comprising foams. To form foam efficiently, the protein needs to adsorb rapidly at the air–liquid interface during the transient stage of foam formation. The adsorption of proteins at interfaces is controlled by three processes: transport from bulk solution to the interface, penetration into the surface layer, and reorganization of the protein structure in the adsorbed layer (17). Foaming capacity is expressed as mL of foam formed per mL of a 0.5% solids dispersion. Foam stability is expressed by k, which is the time for one-half of the liquid to drain from the foam. The smaller k is, the more stable the foam. The rate of foaming is a measure of speed of foam formation. Foaming capacities, stabilities, and rates for the different soy protein products are shown in Table 5.

The EWSPC had the lowest values for all three foaming properties mainly because this product is largely composed of insoluble aggregates, which lack the molecular flexibility to efficiently form stable foams. The LCSPC either exceeded or was equivalent to SPI in foaming.

The LFSPC prepared from HS/LS soybeans extracted at pH 8.5 had lower FC, formed more stable foams, and was slower to form foams compared with the LFSPC extracted at pH 7.5, but these differences were significant only at $P < 0.1$ and insignificant at $P < 0.05$. The higher stability of the LFSPC extracted at pH 8.5 was attributed to its higher solubility and surface hydrophobicity. Both properties are fundamental for foam stabilization. The LFSPC prepared from IA2020 soybeans extracted at pH 7.5 had significantly higher FC, formed foams more quickly, and had a similar foaming stability compared with the LFSPC extracted at pH 8.5.

TABLE 6
Dynamic Viscosity Properties of Protein Ingredients Prepared from Normal and HS/LS Soybeans[a]

Soybeans/protein product	Flow consistency index (mPa·s)	Flow behavior index (dimensionless)
IA2020 soybeans		
LFSPC, pH 7.5	0.004[c]	0.975[a]
LFSPC, pH 8.5	0.008[c]	0.923[a]
SPI	0.151[a]	0.728[c]
EWSPC	232*	0.561*
HS/LS soybeans		
LFSPC, pH 7.5	0.004[c]	0.972[a]
LFSPC, pH 8.5	0.007[c]	0.947[a]
SPI	0.070[b]	0.811[b]
EWSPC	470*	0.660*
LSD	0.045	0.055

[a]$n = 3$. Means within a column followed by different superscripts are significantly different at $P < 0.05$. *Samples not included in statistical analysis. For abbreviations see Table 1.

The LCSPC prepared from HS/LS soybeans had significantly higher FC, K, and V_i than the traditional soy protein ingredients. The protein fractions prepared from HS/LS soybeans had similar or superior foaming properties, with the exception of the foaming rate for IA2020 SPI that was significantly higher than for the SPI prepared from HS/LS soybeans. Similar to emulsion stability, the higher content of β-conglycinin probably accounted for the improved foaming properties of the LCSPC prepared from HS/LS soybeans.

Dynamic viscosity. Dynamic viscosity results are shown in Table 6 for dispersions at the same solids level (10%). Data for EWSPC were not included in the statistical analysis because the correlation coefficient for the data in the power-law regression analysis was <0.8. The readings for these products were not consistent because slurry sampling was highly variable due to poor solubility. These samples had suspended particles that probably interfered with viscosity readings. The low correlation coefficients introduced sufficient variability that we could not compare the rest of the products among themselves. Still, the power-law model was the best fit for the EWSPC data. The correlation coefficients for the LCSPC and SPI were >0.998. The LFSPC extracted at pH 7.5 had a similar flow consistency index (k) and flow behavior index (n) to those of LFSPC extracted at pH 8.5. Both flours behaved the same.

The LCSPC had lower viscosity and flow behavior and were more like a Newtonian fluid than SPI. We attributed this observation to the lower protein content of the LCSPC. The rheological behaviors of soy protein dispersions are highly sensitive to protein concentration (18). To compare the LCSPC to SPI at the same protein concentration, we dispersed the LCSPC at the same protein concentration as in the original 10% SPI dispersion; the resulting slurries of LCSPC contained more solids. The k and n values were 0.0322 and 0.838 for the LFSPC extracted at pH 8.5, and 0.010 and 0.938 for the LFSPC extracted at pH 7.5, respectively. The LCSPC, at the same protein concentration, had lower dynamic viscosity values than SPI prepared from IA2020 soybeans (LSD = 0.031 and 0.019 for k and n, respectively). We attributed these differences to less denaturation of the glycinin component and a similar or higher degree of denaturation in the β-conglycinin component of the LCSPC than in the SPI. In addition, the LCSPC had higher ash (salt) contents than the SPI (3). Higher salt concentrations reduce apparent viscosities of soy protein dispersions, probably due to increased protein solubilities (18). The SPI prepared from HS/LS soybeans had a significantly lower k value and higher n value than the SPI prepared from IA2020 soybeans, which was attributed to the fact that SPI made from IA2020 had more native β-conglycinin than did the SPI prepared from HS/LS. Rickert *et al*. (8) and Bian *et al.* (10) reported that native β-conglycinin dispersions are more viscous than native glycinin dispersions.

These unique and desirable sets of functional properties of the LCSPC make them viable alternative soy protein ingredients that are suitable for industrial applications as food additives and ingredients.

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