Compositional Characteristics of Protein Ingredients Prepared from High-sucrose/Low-stachyose Soybeans

Nicolas A. Deak, Patricia Murphy, and Lawrence A. Johnson*

Department of Food Science and Human Nutrition and Center for Crops Utilization Research, Iowa State University, Ames, Iowa

ABSTRACT: High-sucrose/low-stachyose (HS/LS) soybeans contained lower total concentrations of free sugars (13.3%), less stachyose (0.7%), and more galactinol (0.7%) (galactopyranosyl-myo-inositol) than the control normal soybeans (14.9, 5.1, and 0.2%, respectively). A low-fiber soybean protein concentrate (LFSPC) process was developed, which is especially suited to HS/LS soybeans, by which defatted soy flour is merely extracted with alkali to remove fiber and then neutralized and dried to produce the protein-rich soluble fraction. Two different pH values (7.5 and 8.5) were used in extracting protein, and these LFSPC were compared with traditional ethanol-washed soy protein concentrate (EWSPC) and soy protein isolate (SPI) prepared from both normal and HS/LS soybeans. The LFSPC had slightly lower yields of solids and protein (~70 and ~81%, respectively) than conventional EWSPC (~77 and ~93%, respectively) but much higher than conventional SPI (~42 and ~70%, respectively). The LFSPC prepared from HS/LS soybeans contained significantly ($P < 0.05$) more protein (~66% protein content) than LFSPC prepared from normal soybeans (~63%). Total isoflavone contents of the LFSPC (~12 μmol/g) were significantly higher than for EWSPC (~1.5 Ìmol/g) or SPI (~10 μmol/g). The LFSPC prepared from HS/LS soybeans contained higher sugar contents (~15%) than either traditional EWSPC (~2.5%) or SPI (~1.5%); but the sums of stachyose and raffinose were only ~1% for the LFSPC compared with ~1% for EWSPC and 0.5% for SPI prepared from normal soybeans.

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KEY WORDS: Isoflavones, oligosaccharides, protein, raffinose, soybeans, soybean sugars, soy protein concentrate, soy protein isolate, stachyose.

Soybeans are an important world commodity because of their wide range of geographical adoption, unique chemical composition (i.e., high protein content), high nutritional value, unique potential health benefits, and versatile uses. There are several constraints, however, associated with using soybeans and soy protein ingredients in human food, including beany flavor, low oxidative stability of soybean oil, and the presence of protease inhibitors and flatulence-causing oligosaccharides (1). Consequently, only a small portion of the annual soybean production is used for human food.

Excessive accumulation of intestinal gas, i.e., flatulence, has been a significant limiting factor to using soybeans and soy protein ingredients in food and feed. Flatulence results from the presence of significant amounts of α-linked oligosaccharides, mainly raffinose and stachyose in normal soybeans. These two nonreducing sugars are composed of one or two galactose units linked to sucrose. Humans and other monogastric animals lack α-1,6-galactosidase in their intestinal mucosa to hydrolyze these sugars. When ingested, these soluble sugars are not absorbed, do not contribute metabolizable energy, and pass into the lower intestinal tract where they are metabolized by intestinal microflora, which possess the enzyme, leading to gas production (2).

The elimination of these unwanted oligosaccharides from soy protein ingredients has been largely accomplished in the past through processing, but more recently genetic control offers promise. Soy protein concentrates (SPC) are widely used in the food industry; and three processes, differing in the method used to render the protein insoluble in the extracting solvent, are used to prepare them. During processing, however, the protein is denatured, which compromises its functionality and applications. The three traditional processes include washing with aqueous ethanol, washing with acid (at pH 4.5), and washing with water (pH ~6.7) after moist heating (1). All of these processes have the objective of extracting the soluble sugars and ash mineral components from the protein-fiber fraction of soybean meal to obtain SPC containing at least 65% protein. The most widely used method is aqueous ethanol extraction because better flavor results. All of these processes produce a by-product of soy molasses, which poses disposal problems. During ethanol washing, significant amounts of potentially healthy isoflavones are lost into the molasses (sometimes recovered by additional process steps) and protein is denatured.

There is considerable natural variation in raffinose (0.1–0.9%) and stachyose (1.4–4.1%) contents among commonly grown varieties of soybeans (3). It is now possible to use molecular biology to modify soybeans genetically to shift the sugar composition to elevated sucrose and reduced oligosaccharide contents (4). Using defatted meal from these high-sucrose/low-stachyose (HS/LS) soybeans enables new methods to prepare soy protein ingredients. The U.S. patents of Crank and Kerr (4) and Johnson (5) disclosed new, simpler methods based on removing the fiber while retaining the sugars to produce low-fiber soy protein concentrates (LFSPC) by merely extracting with alkali and then neutralizing and spray-drying the protein-rich extract. These new products have not been systematically characterized and evaluated, but one expects

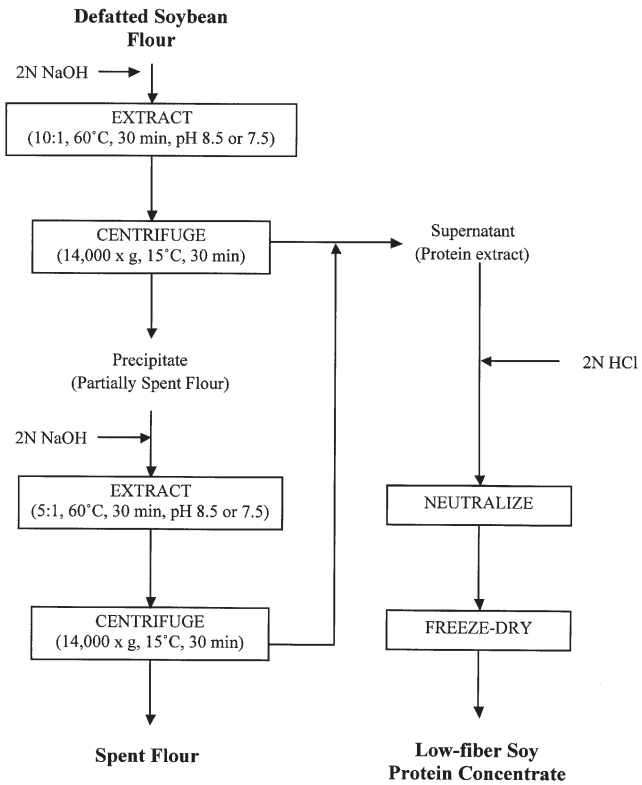
*To whom correspondence should be addressed at Center for Crops Utilization Research, 1041 Food Sciences Bldg., Iowa State University, Ames, IA 50011-1061. E-mail: ljohnson@iastate.edu

very different compositions and functionalities than are offered by today's soy protein ingredients. The objective of the present study was to characterize and compare these LFSPC with the traditional soy protein ingredients, ethanol-washed soy protein concentrate (EWSPC) and soy protein isolate (SPI).

EXPERIMENTAL PROCEDURES

Materials. Air-desolventized, hexane-defatted white flakes from a commonly grown variety of normal soybeans (IA2020 variety, 1999 harvest), which was used as the control flour, and from HS/LS soybeans (2 HS Soybeans, Low Stachyose, Lot-980B0001 OPTIMUM; Pioneer-DuPont, Johnston, IA) were prepared in the pilot plant at the Center for Crops Utilization Research by using a French Oil Mill Machinery Co. extractor-simulator (Piqua, OH). The flakes were milled by using a Krups grinder (Distrito Federal, Mexico) until 100% of the material passed through a 50-mesh screen. Small quantities (~10 g) were ground at any one time to preserve the native protein state. The flours were stored in sealed containers at 4°C until used.

LFSPC preparation. LFSPC were prepared in the laboratory by simulating the methods in the Crank and Kerr patent (4), in which protein is extracted at pH 7.5, and methods in the Johnson patent (5), in which protein is extracted at pH 8.5 (Scheme 1). About 100 g of defatted soy flour was extracted with de-ionized water at a 10:1 water/flour ratio, the pH was adjusted to 7.5 or 8.5 with 2 N NaOH, and the resulting slurry was stirred for 30 min at 60ºC. After centrifuging at 14,300 × *g* for 30 min at room temperature, a protein extract was obtained and the insoluble fiber residue was re-extracted with additional de-ionized water at 5:1 water/insoluble fiber ratio. The pH was adjusted to the pH of the first extraction, and the slurry was stirred for 30 min at 60ºC. After centrifuging at 14,300 × *g* for 30 min at room temperature, the second protein extract was combined with the first protein extract, and the insoluble fiber was sampled and discarded. The combined extract was adjusted to pH 7.0 with 2 N HCl and freeze-dried. The dry protein products (LFSPC) were stored in sealed containers until used. These procedures were replicated three times with each flour.



SCHEME 1

EWSPC preparation. About 100 g of defatted soy flour was extracted with 60% ethanol/40% de-ionized water at a 10:1 solvent/flour ratio and 40ºC, and the resulting slurry was stirred for 30 min in sealed containers to avoid ethanol evaporation. After centrifuging at 14,300 × *g* for 30 min at room temperature, SPC was obtained as the residual solids, and the extract (supernatant, soy molasses), containing primarily soluble sugars, was sampled and the remainder was discarded. The SPC was air-desolventized in a fume hood at 25ºC for 24 h. The samples were then freeze-dried and stored in sealed containers until used. These procedures were replicated three times with each flour.

SPI preparation. About 150 g defatted soy flour was extracted with de-ionized water at a 10:1 water/flour ratio, the pH was adjusted to 8.5 with 2 N NaOH, and the resulting slurry was stirred for 30 min at 60°C. After centrifuging at 14,300 × *g* for 30 min at room temperature, a protein extract was obtained and the insoluble fiber residue was sampled and discarded. The protein extract was adjusted to pH 4.5 with 2 N HCl and centrifuged as before. A protein curd was obtained as the precipitate, and the supernatant (whey) was sampled, with the remainder being discarded. The curd was redissolved in de-ionized water, and 2 N NaOH was added to achieve pH 7 with approximately 10% solids content. The protein slurry was freeze-dried and stored in sealed containers until used. These procedures were replicated three times with each soy flour.

Proximate analyses and mass balances. The nitrogen contents of the soy flours and each product and waste stream were determined by using the combustion or Dumas method (6) and a Rapid NIII Analyzer (Elementar Americas, Inc., Mt. Laurel, NJ). These values were converted to Kjeldahl nitrogen concentration by using the conversion equation of Jung *et al.* (7) of $y = -.00536 + 0.97188x$, where y = converted Kjeldahl nitrogen value and x = nitrogen value from Dumas method. The conversion factor used to convert percentage of nitrogen to protein content was 6.25. Moisture content was determined by oven-drying for 3 h at 130°C (8). Ash and crude fiber contents were determined by using AACC (9) and AOCS standard methods (10), respectively. Protein dispersibility index (PDI) was determined by Silliker Laboratories (Minnetonka, MN). Mass balances of solids and protein were performed for all products, and yields were determined. All measurements were replicated at least three times and means reported.

Protein compositions. Urea-SDS-PAGE was performed by using methods of Rickert *et al.* (11) to quantify the protein

composition profiles of the protein products. Lipoxygenase and soy storage protein bands were identified by using a pre-stained SDS-PAGE MW standard, low range (Bio-Rad Laboratories, Hercules, CA). Glycinin and β-conglycinin subunit bands were confirmed by using purified standards produced according to methods of O'Keefe *et al*. (12). Amounts of all unidentified bands were summed and reported as "others." Densitometry was carried out by using the Kodak 1D Image Analysis, version 3.5 (Kodak, Rochester, NY) on scanned images produced by a Biotech image scanner (Amersham Pharmacia, Piscataway, NJ). SDS-PAGE results were calculated as % composition = [(band or sum of subunit bands)/(sum of all bands)] × 100. All measurements were replicated at least four times and means reported.

Sugar compositions. Samples (approximately 2 g) were extracted with 50 mL 1:1 denatured ethanol/water. The extracts were then filtered through a 0.45-µm polytetrafluoroethylene (PTFE) syringe filter (Alltech Associates, Deerfield, IL) and analyzed by HPLC. The HPLC column was an Interaction CHO-620 (Alltech Associates) with water containing a small amount of calcium disodium EDTA as the mobile phase at 0.5 mL/min flow rate. The column was operated at 80°C. The Waters 2410 refractive index detector (Waters Corporation, Milford, MA) was operated at 64× sensitivity. The injection volume was 20 µL. TurboChrom data system software was used for data collection and report generation. Peaks identified by using standards were stachyose, raffinose, sucrose, galactinol, glucose, galactose, and fructose. Samples were run in triplicate and means reported.

Isoflavone compositions. Isoflavones were extracted and analyzed by using HPLC and methods of Murphy *et al*. (13). About 2.5 g of freeze-dried sample was extracted with 10 mL of acetonitrile, 2 mL of 0.1 N HCl, and about 10 mL of water, by stirring this slurry for 2 h at 25ºC. After filtering, the samples were dried in a rotary evaporator at <30°C. The dry residue was redissolved in 80% HPLC-grade methanol. Aliquots of these extracts were filtered and analyzed by HPLC within 10 h of extraction. Total isoflavone contents were adjusted for M.W. differences and expressed as aglucon contents of the individual isoforms (µg/g). These adjusted contents were used for calculating yields, where % yield in a given product = [(total isoflavone concentration in a given product * mass of the given product)/(total isoflavone concentration in the starting flour * initial mass of flour)] *100. Molar concentrations were used for determining isoflavone profiles. Samples were run in duplicate and means reported.

Statistical analysis. The data were analyzed by ANOVA and General Linear Model. Least significant differences (LSD) were calculated at $P < 0.05$ to compare treatment means using the SAS system (version 8.2; SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Compositions of soy flours. The HS/LS soy flour contained slightly less total sugar, much less flatulence-causing sugars, and more total isoflavones, and had a similar PDI compared with soy flour prepared from normal soybeans. The defatted flour prepared from HS/LS soybeans contained 58.3% protein, 13.3% total sugars (0.7% stachyose, 1.0% raffinose, 10.5% sucrose, 0.7% galactinol), and 2,657 µg/g total isoflavones, and had 95.0 PDI, whereas the flour prepared from IA2020 soybeans (control normal soybeans) contained 57.3% protein, 14.9% total sugars, 5.1% stachyose, 1.4% raffinose, 7.5% sucrose, 0.2% galactinol, and 1,922 µg/g total isoflavones, and had 93.8 PDI.

Proximate compositions and yields of protein products. The LFSPC prepared from HS/LS soybeans had protein contents exceeding the critical industry minimum limit of 65% protein (dry basis) and very low crude fiber contents, whereas those prepared from IA2020 (control, normal) soybeans did not quite meet the critical minimum protein content because of higher sugar contents (Table 1). Both LFSPC (extracted at pH 8.5 and 7.5) produced from IA2020 soybeans contained significantly less protein and isoflavones, significantly more total sugar, and

TABLE 1
Yields and Compositions of Protein Ingredients Prepared from Normal and High-sucrose, Low-stachyose Soybeans (%, dry basis)[a]

	Composition					Yield		
Soybeans/product	Protein (%)	Sugar (%)	Ash (%)	Crude fiber (%)	Isoflavone (µg/g)	Solids (%)	Protein (%)	Isoflavone (%)
IA2020 soybeans								
LFSPC, pH 7.5	62.3^{d}	19.1^{a}	8.0^{c}	0.3^{c}	2992^{a,b}	70.4^{c}	81.4^{d}	89.6^{a}
LFSPC, pH 8.5	62.7^{d}	18.9^{a}	8.8^{a}	0.4^{c}	2880^{b}	71.5^{c}	82.3^{d}	87.1^{b}
EWSPC	70.0^{b}	2.9^{c,d}	5.7^{e}	3.4^{b}	412^{d}	76.1^{b}	92.4^{b}	16.3^{f}
SPI	91.3^{a}	1.8^{d}	4.2^{g}	0.3^{c}	2570^{c}	40.7^{g}	69.7^{g}	54.4^{d}
HS/LS soybeans								
LFSPC, pH 7.5	66.6^{c}	14.7^{b}	8.4^{b}	0.3^{c}	3092^{a}	67.4^{e}	79.5^{e}	78.4^{c}
LFSPC, pH 8.5	66.3^{c}	14.7^{b}	8.7^{a}	0.2^{c}	3087^{a}	69.1^{d}	84.0^{c}	80.0^{c}
EWSPC	69.4^{b}	2.2^{c}	6.0^{d}	4.4^{a}	416^{d}	78.4^{a}	94.8^{a}	12.2^{g}
SPI	92.1^{a}	1.3^{d}	4.5^{f}	0.3^{c}	3129^{a}	42.4^{f}	71.6^{f}	49.9^{e}
LSD	1.4	0.9	0.2	0.3	176	1.3	1.2	1.8

[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, normal soybeans; LFSPC, low-fiber soy protein concentrate prepared by extracting with alkali and then neutralizing and spray-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 at $P < 0.05$.

TABLE 2
Protein Compositions of Protein Ingredients Prepared from Normal and HS/LS Soybeans (% of total protein)[a]

Soybeans/product	Lipoxygenase	β-Conglycinin	Glycinin	Others
IA2020 soybeans				
Flour	5.37[a]	30.08[c]	50.22[b]	14.33[a,b]
LFSPC, pH 7.5	3.90[c]	30.49[c]	52.22[a,b]	13.39[b]
LFSPC, pH 8.5	3.07[d]	32.41[b,c]	54.23[a]	10.29[d,e]
EWSPC	4.63[b]	31.42[b,c]	52.93[a]	11.02[c,d]
SPI	3.92[c]	33.52[b]	52.73[a]	9.83[e]
HS/LS soybeans				
Flour	5.94[a]	29.01[c]	50.51[b]	14.54[a]
LFSPC, pH 7.5	3.84[c]	36.80[a]	48.60[b]	10.76[c,d,e]
LFSPC, pH 8.5	3.72[c]	36.85[a]	48.73[b]	10.69[c,d,e]
EWSPC	3.37[c,d]	31.38[b,c]	54.99[a]	10.26[d,e]
SPI	2.86[d]	31.81[b,c]	53.79[a]	11.54[c]
LSD	0.61	2.29	3.66	1.12

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

similar ash and crude fiber contents compared with LFSPC produced from HS/LS soybeans. These differences were attributed to the IA2020 flour having lower protein and isoflavone contents and higher sugar content. The proximate compositions of the LFSPC were similar except for ash contents, which were slightly higher for the LFSPC extracted at pH 8.5 for both soybean varieties owing to the salt produced during neutralization. The LFSPC also had much higher total sugar contents compared with the SPI and EWSPC as had been expected because the sugars were extracted along with the protein. The LFSPC had crude fiber contents similar to those of SPI and significantly lower than those of EWSPC. Most of the isoflavones were extracted from the insoluble protein when producing EWSPC, and a significant amount of isoflavones was lost to the whey in producing SPI (14). The isoflavone contents of the LFSPC were significantly higher than those of the traditional soy protein ingredients because the complete aqueous extracts were dried when preparing LFSPC.

Significantly higher yields of solids and protein were achieved for the LFSPC compared with SPI, and lower yields of solids and protein compared with EWSPC. In general, significantly higher yields of protein products were achieved with HS/LS soybeans than with IA2020 soybeans, probably owing to the higher protein content of the HS/LS soy flour. The isoflavone yields were also significantly higher for the LFSPC compared with traditional soy protein ingredients. Higher yields of isoflavones were recovered in the protein products prepared from IA2020 soybeans, but these products had lower isoflavone concentrations in the finished protein products. The latter was attributed to the significantly lower isoflavone content of the IA2020 flour.

The LFSPC produced from HS/LS soy flour and extracted at pH 8.5 had higher yields of solids and protein compared with LFSPC extracted at pH 7.5. On the other hand, the yields of solids and protein for the LFSPC prepared from IA2020 soy flour at the two extraction pH values were not significantly different (Table 1). Total isoflavone yields were not significantly different for the LFSPC prepared from HS/LS soy flour, whereas the total isoflavone yields for the LFSPC extracted at pH 8.5 were higher than for the LFSPC extracted at pH 7.5 and made from IA2020 soybeans.

The LFSPC prepared from IA2020 soybeans yielded significantly higher amounts of solids and isoflavones, but the LFSPC extracted at pH 8.5 had lower protein yield compared with LFSPC prepared from HS/LS soybeans. The isoflavone yields were not significantly different.

Protein compositions. The protein component profiles of the two flours were similar (Table 2). There were no differences in the protein component profiles for the two LFSPC; extraction pH did not affect the protein profiles of the LFSPC prepared from HS/LS soybeans. The protein profiles of LFSPC prepared from IA2020 soybeans were significantly different from those prepared from HS/LS soybeans. The LFSPC prepared from IA2020 soybeans contained significantly less β-conglycinin and more glycinin.

The protein profiles of all protein products were different from those of the starting soy flours. The protein profiles of the LFSPC differed from those of traditional soy protein ingredients. This differential partitioning of the proteins was attributed to different extents of solubilizing each protein. The EWSPC prepared from IA2020 soybeans contained significantly more lipoxygenase compared with the other protein products. The LFSPC contained more β-conglycinin and less glycinin than either EWSPC or SPI. This increased concentration in β-conglycinin may affect the functional properties of these ingredients.

The protein products prepared from HS/LS soybeans also had different protein profiles from the products prepared from IA2020 soybeans. The LFSPC prepared from HS/LS soybeans had higher ratios of β-conglycinin to glycinin than LFSPC from IA2020 soybeans. SPI and EWSPC prepared from HS/LS soybeans contained less lipoxygenase than SPI prepared from IA2020 soybeans. The ratios of β-conglycinin to glycinin for SPI and EWSPC were the same for both flours.

Sugar compositions. The protein products prepared from IA2020 soybeans contained more sugars than the same products prepared from HS/LS soybeans (Table 1). The SPI prepared from HS/LS soybeans had one-tenth of the amount of stachyose, six times as much galactinol, and similar amounts of the other sugars (Table 3) compared with SPI prepared from IA2020 soybeans.

The LFSPC contained many more sugars than the traditional soybean protein ingredients; however, the stachyose contents of the LFSPC were similar to that of SPI and less than that of EWSPC (Table 1). The raffinose contents of the LFSPC were slightly higher than those of the traditional soy protein ingredients. The LFSPC were about 10-fold higher in sucrose and 30-fold higher in galactinol contents than the SPI and EWSPC.

The LFSPC prepared from HS/LS soybeans had very different sugar profiles owing to compositional differences of the soy flours. The sugar profile of the LFSPC prepared by extracting HS/LS soy flour at pH 8.5 was not significantly different from that of the LFSPC extracted at pH 7.5 from the same soy flour (Table 3). The sugar profiles of the same protein products prepared from IA2020 soybeans were different, with much higher contents of stachyose (over 13 times more) and about 16% higher raffinose content, from the same products produced from HS/LS soybeans. The LFSPC prepared from IA2020 soybeans contained slightly less sucrose, about one-seventh as much galactinol, and more glucose and less fructose than LFSPC produced from HS/LS soybeans.

In October 1999, the U.S. Food and Drug Administration (FDA) approved a health claim for soy protein and soy protein-containing products. To meet the requirements for this health claim, foods must contain 6.25 g soy protein per serving (14). Parsons *et al.* (15) compared the total metabolizable energy of three conventional soybean meals and five low-oligosaccharide soybean meals fed to roosters and concluded that the total metabolizable energy of low-oligosaccharide soybean meals was significantly higher. Suarez *et al.* (16) compared gas production and gaseous symptoms in healthy human subjects fed either normal or HS/LS soybeans, and concluded that those subjects fed soy flour low in oligosaccharides produced less gas than those fed conventional soy flour. Both studies (15,16) used soybean materials that had similar sugar profiles to our soybean flours. Based on these studies and the health claim on soy protein, we calculated the amounts of ingredients that would be needed per serving to meet FDA's requirements to be 10.7 g for HS/LS soy flour, 9.4 g for LFSPC made from HS/LS soy flour, 8.9 g for EWSPC made from normal soybeans, and 6.85 g for SPI made from normal soybeans. Based on the sugar profile, we calculated the amount of indigestible sugars (stachyose + raffinose + galactinol; we assumed galactinol is indigestible since no data could be found) that each of these servings would contain is 0.25 g of indigestible sugar/serving for HS/LS soy flour and 0.16 g for LFSPC, 0.11 g for EWSPC, and 0.04 for SPI made from HS/LS soybeans. When these same calculations were made for normal soy flour, the amount of indigestible sugar increased to 0.72 g/serving. LFSPC made from HS/LS soybeans contained higher amounts of indigestible sugars compared with traditional soy protein ingredients (45% more than EWSPC and 4 times more than SPI), but these amounts were significantly lower than for normal soy flour (about 78% less). These LFSPC ingredients have reduced amounts of indigestible sugars and can replace some traditional soy protein ingredients without causing intestinal gas.

Isoflavone compositions. The isoflavone component profiles of the soy flours and protein products are shown in Table 4. The isoflavones commonly found in soybeans and soy protein products are genistein, daidzein, and glycitein, which occur in four forms: the aglucon, the β-glucoside, the malonyl-β-glucoside, and the acetyl-β-glucoside. Of these four isoforms, the β-glucosides and the malonyl-β-glucosides predominant in soybeans (13) and the isoflavone profile and isoforms distribution are altered during processing (17,18).

The isoflavone contents and profiles of the soy flours were significantly different between the two types of soybeans. Soy

TABLE 3
Sugar Compositions Protein Ingredients Prepared from Normal and HS/LS Soybeans (% dry basis)[a]

Soybeans/product	Stachyose	Raffinose	Sucrose	Galactinol	Glucose	Galactose	Fructose
IA2020 soybeans							
Flour	5.07^{b}	1.38^{a}	7.48^{d}	0.16^{c}	0.58^{a}	0.09^{a}	0.11^{c}
LFSPC, pH 7.5	6.17^{a}	0.77^{c}	11.56^{b}	0.09^{d,e}	0.49^{b}	0.00^{d}	0.08^{d}
LFSPC, pH 8.5	6.08^{a}	0.75^{c}	11.45^{b}	0.10^{d}	0.46^{c}	0.00^{d}	0.07^{d,e}
EWSPC	0.90^{c}	0.22^{e}	1.55^{e,f}	0.02^{f}	0.08^{f}	0.07^{b}	0.04^{e}
SPI	0.47^{d}	0.05^{f}	1.16^{f}	0.01^{f}	0.05^{g}	0.00^{d}	0.05^{c,d}
HS/LS soybeans							
Flour	0.71^{c,d}	0.98^{b}	10.54^{c}	0.71^{a}	0.23^{d}	0.00^{d}	0.08^{d}
LFSPC, pH 7.5	0.44^{d,e}	0.62^{d}	12.65^{a}	0.62^{b}	0.11^{e}	0.00^{d}	0.29^{a}
LFSPC, pH 8.5	0.45^{d}	0.66^{d}	12.60^{a}	0.61^{b}	0.09^{e,f}	0.00^{d}	0.25^{b}
EWSPC	0.07^{e,f}	0.10^{f}	1.81^{e}	0.09^{d,e}	0.03^{g,h}	0.05^{c}	0.05^{e}
SPI	0.04^{f}	0.04^{f}	1.07^{f}	0.06^{e}	0.01^{h}	0.00^{d}	0.07^{d,e}
LSD	0.38	0.07	0.54	0.03	0.03	0.01	0.03

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

TABLE 4
Isoflavone Compositions of Protein Ingredients Prepared from Normal and HS/LS Soybeans (μmol/g)[a]

Soybeans/product	Din	MDin	AcDin	Dein	Glyin	MGly	Glyein	Gin	MGin	AcGin	Gein	Total
IA2020 soybeans												
Flour	0.73^{f}	2.18^{c}	0.05^{c}	0.13^{d}	0.22^{d}	0.25^{b,c}	0.00^{e}	1.01^{f}	2.44^{c}	0.08^{f}	0.10^{c}	7.20^{d}
LFSPC, pH 7.5	1.58^{c,d}	3.08^{a}	0.09^{b}	0.61^{a}	0.35^{b}	0.40^{a}	0.11^{a}	1.47^{e}	2.76^{c}	0.11^{d}	0.67^{a}	11.24^{a,b}
LFSPC, pH 8.5	2.60^{a}	1.87^{d}	0.06^{c}	0.44^{b}	0.50^{a}	0.27^{b}	0.09^{b}	2.57^{c}	1.79^{e}	0.09^{e}	0.57^{a,b}	10.85^{b}
EWSPC	0.19^{g}	0.36^{e}	0.04^{d}	0.09^{d}	0.06^{e}	0.06^{e}	0.03^{d}	0.25^{g}	0.34^{f}	0.07^{g}	0.07^{c}	1.54^{e}
SPI	1.70^{c}	1.71^{d}	0.08^{b}	0.40^{b,c}	0.31^{c}	0.23^{c}	0.07^{b,c}	2.44^{c}	2.05^{d}	0.15^{c}	0.51^{a,b}	9.65^{c}
HS/LS soybeans												
Flour	1.51^{c,d}	2.95^{b}	0.07^{c}	0.18^{d}	0.34^{b,c}	0.39^{a}	0.04^{d}	1.62^{d,e}	2.65^{c}	0.09^{e}	0.13^{c}	9.97^{c}
LFSPC, pH 7.5	1.24^{e}	3.17^{a}	0.11^{a}	0.27^{c}	0.22^{d}	0.26^{b,c}	0.06^{c}	1.79^{d}	3.89^{a}	0.16^{b}	0.40^{b}	11.59^{a}
LFSPC, pH 8.5	2.18^{b}	2.12^{c}	0.06^{c}	0.28^{c}	0.31^{c}	0.18^{d}	0.05^{c,d}	3.21^{a}	2.70^{c}	0.11^{d}	0.41^{b}	11.60^{a}
EWSPC	0.12^{g}	0.39^{e}	0.04^{d}	0.06^{d}	0.04^{e}	0.05^{e}	0.00^{e}	0.22^{g}	0.48^{f}	0.08^{f}	0.06^{c}	1.56^{e}
SPI	1.45^{d}	2.26^{c}	0.10^{a}	0.30^{c}	0.21^{d}	0.24^{b,c}	0.06^{c}	2.93^{b}	3.47^{b}	0.21^{a}	0.49^{b}	11.72^{a}
LSD	0.20	0.20	0.01	0.13	0.03	0.04	0.02	0.27	0.21	0.01	0.18	0.67

[a]$n = 3$. Means within a column followed by different superscripts are significantly different at $P < 0.05$. Din denotes daidzin; MDin, malonyldaidzin; AcDin, acetyldaidzin; Dein, daidzein; Glyin, glycitin; MGly, malonylglycitin; Glyein, glycitein; Gin, genistin; MGin, malonylgenistin; AcGin, acetylgenistin; and Gein, genistein. For other abbreviations see Table 1.

flour prepared from HS/LS soybeans contained about 38% more total isoflavones than did soy flour prepared from IA2020 soybeans. Because of this difference, we converted the data in Table 4 to percentages of the total isoflavone contents to be able to compare the conversion and partitioning of isoflavone isoforms. The defatted flour prepared from HS/LS soybeans contained 47.5% daidzein, 45.0% genistein, and 7.7% glycitein, whereas the flour prepared from IA2020 soybeans contained 42.9% daidzein, 50.4% genistein, and 6.5% glycitein. Both flours contained about 95% glucosides plus malonylglucosides and only 5% of the other two isoforms. The aglucon isoflavone contents for both flours were about 3%.

The extraction pH used to prepare the LFSPC did not significantly affect isoflavone extraction, and the LFSPC prepared from both soybean types contained about 40% daidzein, 55% genistein, and 5% glycitein, with similar total yields and concentrations. The isoform distribution, however, was significantly affected. The LFSPC extracted at pH 8.5 contained significantly less malonylglucosides (43.2%) and acetylglucosides (1.5%), and higher amounts of glucosides (49.1%) than the same products extracted at pH 7.5 (63.2% malonylglucosides, 2.3% acetylglucosides, and 28.0% glucosides). The conversion from malonylglucoside to the glucoside isoform has been previously reported (17,18), and alkali extraction significantly favors conversion. The aglucon isoform contents for both extraction pH values significantly increased, from 3.5% in the flour prepared from HS/LS soybeans to 6.4% in the LFSPC. This result was partly attributed to the action of native soybean β-glucosidases during the extraction step (18).

When comparing the LFSPC prepared from HS/LS soybeans with those prepared from IA2020 soybeans, we observed different isoflavone profiles. The LFSPC prepared from IA2020 soybeans had similar total daidzein and genistein contents (~46%) and about 8% glycitein. Their isoform distributions followed the same trend as was observed for the HS/LS protein products, but with higher aglucon isoform production (10.1 and 12.4% for the products extracted at pH 8.5 and 7.5, respectively).

The LFSPC had significantly different isoflavone profiles compared with those of traditional soy protein ingredients. The LFSPC prepared from IA2020 soybeans had similar contents of daidzein, genistein, and glycitein, as did the LFSPC prepared from HS/LS soybeans. Apparently, the isoflavones present in the soy flour of normal soybeans were more completely solubilized during extraction than those in HS/LS soy flour. The EWSPC had significantly lower total isoflavone content than the LFSPC, and the distribution was also significantly different. This was not surprising since isoflavones are lost during ethanol washing of soy flour. The isoflavone distribution for LFSPC prepared from IA2020 soybeans was 44.1% daidzein, 47.4% genistein, and 9.7% glycitein. When comparing the isoform distributions, the LFSPC prepared from IA2020 soybeans contained similar amounts glucoside and malonylglucoside in amounts similar to those in LFSPC prepared from HS/LS soybeans when extracted at pH 8.5 (46.1 and 41.4%, respectively), but had significantly more acetylglucoside and aglucon (3.3 and 10.2%, respectively). The EWSPC prepared from IA2020 soybeans had a unique isoform distribution: 49.3% malonylglucosides, 32.5% glucosides, 7.1% acetylglucosides, and 12.3% aglucons. These data indicated that either significant conversion of malonylglucosides to acetylglucosides and aglucons occurred or the ethanol extraction redistributed the native isoflavone profile. The latter reason is more likely since only about 10% of the original soy flour isoflavones were recovered in EWSPC; and the processing temperature of 40°C, ethanol concentration of about 60%, and extraction pH of about 6.8 should have limited the activity of native β-glucosidases, heat conversion, and alkaline hydrolysis.

In general, the protein products prepared from HS/LS soybeans had significantly different isoflavone profiles from the same protein products prepared from IA2020 soybeans. The protein products prepared from IA2020 soybeans had consistently higher aglucon isoform contents (3 to 4 times higher than in the starting soy flour). The protein products prepared from HS/LS soybeans had consistently higher genistein and lower

daidzein contents than the same protein products prepared from IA2020 soybeans, which was not surprising since the protein products made from HS/LS soy flour had higher levels of isoflavones.

Integration of yield and composition data. The LFSPC were low in crude fiber and indigestible sugars and high in minerals and isoflavones. This LFSPC procedure yielded significantly more solids and protein compared with alternative soy protein ingredient processes, and the LFSPC had unique sugar, protein, and isoflavone profiles, and exceeded the critical industrial standard of at least 65% protein. The LFSPC had unique protein profiles, enriched in β-conglycinin, which should affect their functional properties. The LFSPC were not exposed to acid or aqueous ethanol, which denature protein. Therefore, these LFSPC should have unique applications as food ingredients.

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