Preparation of Soy Protein Concentrate and Isolate from Extruded-Expelled Soybean Meals

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ABSTRACT: Soy protein concentrates (SPC) and soy protein isolates (SPI) were produced from hexane-defatted soy white flakes and from two extruded-expelled (EE) soy protein meals with different degrees of protein denaturation. Processing characteristics, such as yield and protein content, and the key protein functional properties of the products were investigated. Both acid- and alcohol-washed SPC from the two EE meals had higher yields but lower protein contents than that from white flakes. Generally, SPC from an acid wash had much better functional properties than those from an alcohol wash. The SPI yield was highly proportional to the protein dispersibility index (PDI) of the starting material, so the EE meal with lower PDI had lower SPI recovery. The protein content in SPI prepared from EE meals was about 80%, which was lower than from white flakes. Nevertheless, SPI from EE meals showed functional properties similar to or better than those from white flakes. The low protein contents in SPC and SPI made from EE meals were mainly due to the presence of residual oil in the final products. SPI made from EE meals had higher concentration of glycinin relative to β-conglycinin than that from white flakes.

Paper no. J10816 in *JAOCS 81*, 713–717 (July 2004).

KEY WORDS: Extruding-expelling, functional property, soy protein concentrate, soy protein isolate.

Soy protein products have become increasingly popular because of their low price, high nutritional quality, and versatile functional properties. Two important soybean protein products are soy protein concentrate (SPC) and soy protein isolate (SPI). SPC is defined as an edible protein product with a protein content of at least 65% on dry weight basis (1), whereas SPI is a product with at least 90% protein on dry weight basis (2). Currently, flash-desolventized solvent-extracted white flakes (typically containing 50% protein) are generally the starting materials for SPC and SPI preparation. Other soybean meals or flours besides white flakes may also be used as starting materials provided that the final products meet protein content specifications and demonstrate desired functional properties.

Soybean meals produced from the extruding-expelling (EE) processing of soybeans may be used as starting materials for SPC and SPI preparation. EE is a mechanical processing technology that allows small-scale production of protein meals having a high oil content and partial recovery of oil. Extrusion, the first step of processing, provides a heat treatment that reduces trypsin inhibitors, permitting the use of the full-fat or defatted protein meals as livestock feed. The extrudate can be pressed by an expeller to partially recover the oil. The protein in the meal typically is extensively heat-denatured by extrusion. Depending on the processing conditions, EE meals with different oil contents and protein denaturation can be achieved (3). Advantages of EE technology include process simplicity, low capital investment, no need for organic solvents, and applicability to identity-preserved (IP) processing due to its flexibility and efficiency in processing small lots of soybeans.

SPC preparation involves insolubilization of the protein to remove soluble sugars. In SPI preparation, proteins are solublized first to remove the insoluble fiber, then they are precipitated to remove soluble sugars. How the yield and functionality of the SPC and SPI will be affected when the proteins in the starting material are heat denatured is unknown. The objectives of this study were to determine the feasibility of preparing SPC and SPI from EE meals and to evaluate the functional properties of these SPC and SPI products in comparison with those produced from defatted, or white, soy flakes.

EXPERIMENTAL PROCEDURES

EE meals and defatted white flakes. An Insta-Pro International Model 2500 extruder and Model 1500 screw press were used to process the dehulled and cracked Stine soybeans into EE meals. The following extruder processing parameters were used: 11-11-6-6 shear lock configuration, double flight screws, and a restriction die opening setting of 3/8 in. (0.94 cm). The temperature in the last segment of the extruder barrel was 132–143°C, and the total residence time was about 20–25 s. EE processing was conducted at Nutriant (Vinton, Iowa). Two EE flours (ground meals), EE35 and EE60, with oil contents of 7.6 and 13.6% and PDI of 35.3 and 62.0, respectively, were prepared. Defatted white flakes (Nutrisoy® PDI of 90) were purchased from ADM (Archer Daniels Midland, Decatur, IL). EE meals and defatted soy flakes were ground into flour using a Fitz Mill® (Model DAS06, The Fitzpatrick Company, Elmhurst, IL) with a 40-mesh screen. To avoid any further heat denaturation of the proteins, care was taken to minimize heat generation during milling. All flours were stored in sealed plastic bags at –20°C before use.

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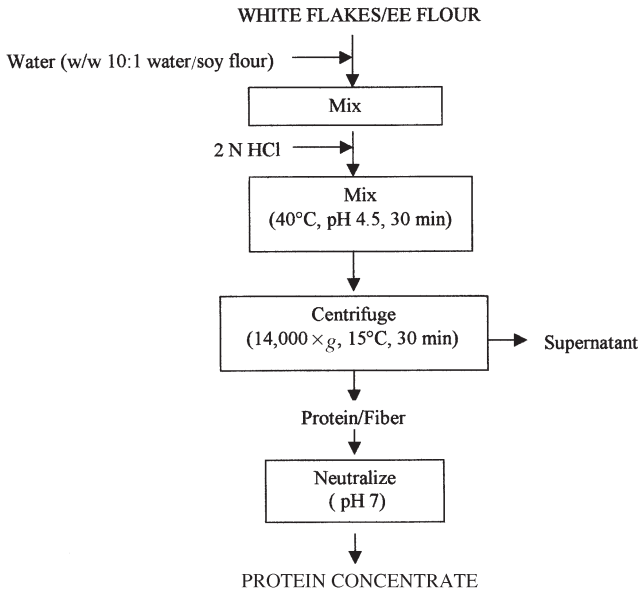

FIG. 1. Procedure for producing soy protein concentrate using the acid-wash method. EE, extruded-expelled.

SPC and SPI preparation. Acid-washed SPC, alcohol-washed SPC, and SPI were prepared at the Center for Crops Utilization Research (CCUR) employing modified protocols of the standard methods (4) (Figs. 1–3). For an acid wash, the standard method requires a ratio of 10–20:1 of water/soybean meals, but a 10:1 ratio was used in this study. The centrifugation *g* force was 14,000 × *g*, at a temperature of 15°C to reduce the protein solubility in the whey. For the alcohol wash, 60% aqueous alcohol was used, compared with 20–80% required in the conventional method. According to Berk (5), on either side

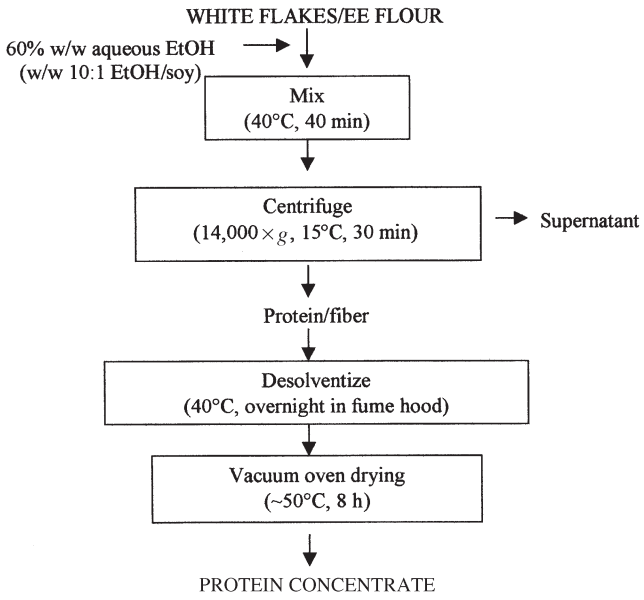

FIG. 2. Procedure for producing soy protein concentrate using the alcohol-wash method. For abbreviation see Figure 1.

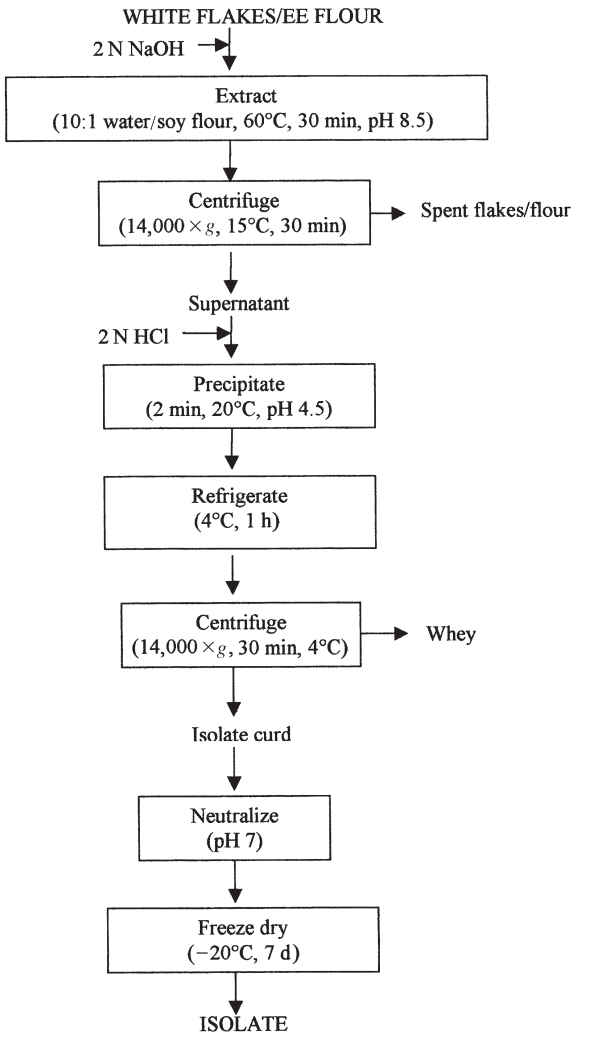

FIG. 3. Procedure for producing soy protein isolate. For abbreviation see Figure 1.

of 60%, soy protein solubility tends to increase. For the SPI procedure, pH 8.5 was used to solublize the soy protein, compared with pH 9–11 in the conventional procedure. According to Berk (5), at pH values higher than 9, cystine tends to be destroyed with the formation of dehydroalanine, which can further react with free ε-amino groups of lysine to produce lysinoalanine, whose toxicological aspect is not fully understood. The supernatant was refrigerated at 4°C after adjusting pH to 4.5, to facilitate the formation of larger and stronger curds. Centrifugation conditions were the same as those used in SPC preparations.

Analytical methods. All concentrations and final data were expressed on a dry weight basis (measured after drying at 130°C for 3 h). All protein contents were measured by the Kjeldahl method (6), and a 6.25 conversion factor was used to calculate protein content. Solid dispersibility and protein dispersibility were measured based on the method of Johnson *et al.* (7). Briefly, a 10% w/w (protein product) dispersion was prepared by stirring for 20 min and cooling for 1 h at 5°C. After centrifugation at 1,050 × *g*, for 5 min at 5°C, the supernatant fraction

was quantified. The dispersible solid was measured by drying and weighing the total solids in the supernatant, and the dispersible protein was measured by using the Kjeldahl method as just discussed to quantify proteins in the supernatant fraction. This protein dispersibility is different from the standard PDI as determined by the AOCS official method (8) in that the measurement conditions are different.

Emulsification capacity was measured based on a method introduced by Swift *et al.* (9). A 25-mL aliquot of a 2% (w/w) dispersion of protein product was placed in a 400-mL plastic beaker. Fully refined soybean oil was added at about 0.5 g/s and mixed with a hand-held mixer. Emulsification capacity was defined as the amount of oil that could be emulsified until the inversion point was observed (9). A fat-soluble dye, Red Fat 7B (Sigma-Aldrich Co., St. Louis, MO) was added in oil at about 2 ppm to enhance the detection of the inversion point.

Foaming property measurement involved a foaming device, which consisted of a graduated glass cylinder with a ceramic frit fused at the bottom. Nitrogen gas was purged at 16.7 mL/s to make a final 300 mL foam from 100 mL of 1% protein sample suspension. Three measurements were made (10): time to reach the final volume (t_f, in s), volume of liquid sample converted to foam at the very end of foaming ($V_{max,}$ in mL), and time required for half of the liquid incorporated into foam to drain back into the liquid fraction ($t_{1/2}$, in s). From these measurements, three foaming parameters were calculated: (i) foaming capacity (FC), an indication of the milliliters of foam formed per milliliter of N_2 purged, and calculated as $FC = 60 \times 300/(16.7 \times t_f)$ in mL/mL units; (ii) K value, which describes foam stability (a higher value indicating lower stability), calculated as $K = 1/(V_{max} \times t_{1/2})$ in $mL^{-1} \times s^{-1}$ unit; and (iii) foaming speed (FS), V_i, which describes the rate of liquid incorporation into foam and is calculated using $V_i = V_{max}/t_f$ in unit of mL/s.

Composition of alcohol-washed SPC and SPI. The total lipid content as determined by acid hydrolysis and crude fiber were determined by Woodson-Tenent Laboratories, Inc. (Des Moines, IA), according to standard AOAC methods (11,12). The total carbohydrate was quantified using the phenol/sulfuric acid method (13). The ratio of β-conglycinin to glycinin in the protein products was evaluated by SDS-PAGE. Scanning densitometry was used to estimate the relative concentration of the various subunits.

Experimental design and data analysis. All analyses were repeated three times except for Kjeldahl measurement, which was duplicated. SPC preparation was performed following a 3 × 2 factorial design, with three protein samples and two wash methods (alcohol and acid). For SPI, three protein samples and only one preparation method were used. Statistical analysis was performed using the General Linear Model procedures of SAS 8.02 (14).

RESULTS AND DISCUSSION

Preparation and functional properties of SPC. The method of SPC preparation, i.e., acid or alcohol wash, and the type of soybean material significantly influenced yield and protein content of SPC, as shown in Table 1. Wash method and sample type had a significant interaction with all quality and functional parameters except for protein content. The alcohol wash resulted in significantly higher SPC and protein yields than the acid wash, especially for white flakes. However, protein contents of SPC from acid wash were statistically higher than those from alcohol wash. These data suggest that the acid wash removed more soluble sugars and recovered relatively more proteins than the alcohol wash. SPC from the two EE meals had a significantly lower protein content than white flakes. The difference was caused mostly by residual oil content in the SPC (Table 2). For example, total lipid contents of alcohol-washed SPC were 3.4, 22.2, and 12.0%, whereas the protein contents were 67.5, 52.2, and 58.8% for white flakes, EE60, and EE35, respectively.

TABLE 1
Acid-Washed and Alcohol-Washed Soy Protein Concentrates (SPC) and Their Functionalities[a]

	Yield (%)		PC	Dispersibility (%)			Foaming property		
	SPC	Protein	(%)	Solids	Protein	EC[a,b]	FS[a,b]	FC[a,b]	*K* value[b]
Acid-washed SPC									
White flakes	72.35	90.93	68.28	38.38	53.72	132.54	0.32	1.45	0.00025
EE60	78.45	95.16	53.86	38.35	47.51	99.00	0.09	0.76	0.00148
EE35	78.16	96.03	60.38	18.88	17.42	127.75	0.20	1.36	0.00097
Alcohol-washed SPC									
White flakes	78.89	98.04	67.53	8.31	5.70	25.76	0.25	1.50	0.00067
EE60	83.98	98.65	52.16	16.08	6.26	19.77	0.21	1.28	0.00086
EE35	81.49	97.56	58.83	12.15	3.93	18.14	0.06	0.55	0.00288
P and LSD values									
Wash	<0.0001	<0.00001	0.0031	<0.0001	<0.0001	<0.0001	0.0054	0.0332	<0.0001
Sample	<0.0001	0.0106	<0.0001	<0.0001	0.0008	0.003	<0.0001	<0.0001	<0.0001
Interaction	0.0001	0.0084	0.5322	<0.0001	0.0009	0.0107	<0.0001	<0.0001	<0.0001
$LSD_{0.05}$ for sample	0.56	1.61	0.97	1.35	3.02	9.93	0.03	0.06	0.0002
$LSD_{0.05}$ for wash	0.45	1.32	0.79	1.10	2.47	8.11	0.03	0.05	0.0002

[a]PC, protein content; EC, emulsification capacity; FS, foaming speed; FC, foaming capacity; EE60, soy flour processed by extruding–expelling and having a protein dispersability index (PDI) of ~60; EE35, same as EE60 except PDI is ~35.
[b]EC: g soybean oil/25 mL 2% slurry; FS: mL/s; FC: mL/mL; *K* value: $mL^{-1} \cdot s^{-1}$.

TABLE 2
Composition of Starting Materials, Alcohol-Washed SPC, and Soy Protein Isolates (SPI)

	Protein (%)	Total carbohydrate (%)	Crude fat (%)	Crude fiber (%)	Ratio of β conglycinin to glycinin
Starting materials					
White flakes	54.34	25.79	3.07	3.94	—[a]
EE60	44.40	23.26	16.73	3.96	—
EE35	49.14	24.01	9.84	4.16	—
Alcohol-washed SPC					
White flakes	67.53	16.17	3.40	4.39	—
EE60	52.16	15.78	22.23	5.17	—
EE35	58.83	17.02	11.96	5.54	—
P value	<0.0001	0.81	<0.0001	0.008	—
$LSD_{0.05}$	1.59	4.72	0.53	0.58	—
SPI					
White flakes	87.53	5.07	3.24	<0.02	0.72
EE60	80.82	5.08	11.69	<0.02	0.57
EE35	79.61	7.61	9.21	<0.02	0.47
P value	0.002	0.80	0.002	—	—
$LSD_{0.05}$	3.33	1.20	3.31	—	—

[a]Not determined. For abbreviations see Table 1.

Oil in initial EE meals could not be removed by either alcohol or acid washing, resulting in lower protein content in the final SPC products. It is noteworthy that the oil contents measured by the acid hydrolysis method were always higher than those measured by standard total lipid quantification methods, such as the Goldfish or Soxhlet method. For example, oil contents of EE35 and EE60 meals were 7.6 and 13.6% by Soxhlet extraction, but 9.8 and 16.7% by the acid hydrolysis procedure (Table 2).

In contrast to the alcohol wash, protein yields from both EE meals by acid wash were higher, by about 5%, than those from white flakes as a result of heat denaturation of protein during the EE process, which made the protein less soluble in acid. Such differences disappeared in alcohol-washed samples as a result of the strong denaturation power of alcohol.

The solids dispersibility for acid-washed SPC directly related to the PDI of the starting material. Alcohol-washed SPC had significantly lower solids dispersibility than acid-washed SPC, and they did not correlate with the initial PDI as a consequence of protein denaturation by alcohol. The same was true for protein dispersibility. SPC from the acid wash had much higher emulsification capacity than that from the alcohol wash, because the alcohol-denatured protein did not disperse well in either water or oil phases. The emulsification capacity of acid-washed SPC from EE35 was similar to that of the white flakes despite its significantly lower protein content, and its emulsification capacity was significantly higher than that of the EE60. It is possible that with the higher degree of protein denaturation, the more hydrophobic regions were exposed, which might have contributed to a higher emulsification capacity. But alcohol-washed SPC showed an opposite trend, implying that alcohol denaturation of protein is different from heat denaturation. The higher residual oil content in SPC from EE60 may also contribute to its lower emulsification capacity.

The foaming properties of acid-washed and alcohol-washed SPC showed quite different patterns also. Under the same wash method, SPC from white flakes had higher foaming speed, foaming capacity, and foam stability values than the SPC from EE samples. Acid-washed SPC from EE35 had significantly higher foaming speed, capacity, and stability values than that from EE60. However, alcohol-washed SPC from white flakes, EE60, and EE35 showed different trends compared with acid-washed products. For alcohol-washed SPC, product from EE35 had the lowest foaming speed, capacity, and foam stability. This also implies that alcohol and heat denature protein in different manners.

SPI preparation and functional properties. SPI yield, protein yield, and protein content were significantly different among SPI prepared from different materials (Table 3). SPI from samples with higher PDI values had higher yields and protein

TABLE 3
SPI Prepared from Different Materials and Their Functionalities[a]

	Yield (%)		PC	Dispersibility (%)			Foaming property		
	SPC	Protein	(%)	Solids	Protein	EC	FS	FC	*K* value
White flakes	45.48	73.27	87.53	95.69	94.07	248.42	0.63	1.69	0.00011
EE60	33.45	60.89	80.82	100.00	100.00	272.28	0.62	1.67	0.00014
EE35	24.98	40.46	79.61	100.00	100.00	316.42	0.63	1.67	0.00013
P value	<0.0001	<0.0001	0.002	0.035	<0.0001	0.021	0.94	0.064	0.001
$LSD_{0.05}$	0.87	2.78	3.32	3.19	0.75	42.7	0.08	0.02	1.00E-5

[a]For abbreviations and units see Tables 1 and 2.

contents than those from lower-PDI materials. Since the total carbohydrates in SPI from white flakes and EE60 were similar (Table 3), the differences in protein contents were apparently partially due to the residual oil content in the SPI. For EE35, the higher total carbohydrates and total oil together contributed to the lower protein content in SPI compared with that from white flakes. Both SPI yield and protein yield showed a strong linear relationship with PDI of the raw materials: SPI yield (%) = 10.3 × PDI + 14.1, R^2 = 0.99; protein yield (%) = 16.4 × PDI + 25.4, R^2 = 0.98. This shows that denaturation of protein strongly affects the amount of protein that can be extracted into SPI. Although the protein content differences among SPI from white flakes and two EE meals were significant, the difference in SPI between the two EE meals was much smaller than that between white flakes and EE meals (Table 2).

SPI from both EE60 and EE35 meals had solids and protein dispersibilities of 100%. The SPI from white flakes had slightly lower solids and protein dispersibilities, about 96 and 94%, respectively. The difference might be due to a small portion of unstable protein in white flakes that was recovered into SPI but that became insoluble during SPI handling and testing, whereas the corresponding proteins in EE meals never had this chance because they endured a much harsher treatment earlier in the EE process and went with the insoluble fractions during SPI preparation.

Contrary to yield, SPI from the two EE meals had significantly higher emulsification capacities than those from the white flakes. To explain this observation, the ratio of β-conglycinin to glycinin was determined by SDS-PAGE and densitometry analysis (Table 3). The ratio for SPI from white flakes was 0.72, but the ratios were 0.57 for EE60 and 0.47 for EE35. Apparently, EE processing denatured relatively more β-conglycinin than glycinin, resulting in a decreased β-conglycinin-to-glycinin ratio. The lower the PDI value (thus the harsher the EE processing), the lower was the ratio. This is reasonable since the denaturation temperature of β-conglycinin was lower than that of glycinin. For instance, in water solutions, denaturation temperatures were about 70 and 90°C for β-conglycinin and glycinin, as measured by DSC in our own research (Wang, H., L.A. Johnson, and T. Wang, unpublished data). Thus, the EE process resulted in a partial fractionation of β-conglycinin and glycinin during SPI preparation and increased the glycinin fraction in the final SPI. The emulsification capacity of β-conglycinin was 1.5–4.0-fold higher than that of glycinin, as reported by Bian *et al.* (15), and 1.7- or 3.8-fold higher as reported by Rickert *et al.* (16); therefore, we expected to have a lower emulsification capacity for the SPI from EE meals. However, the emulsification capacity of SPI from EE35 was significantly higher than that from white flakes, which was contrary to the expected outcome based on the β-conglycinin and glycinin ratio change. One possible explanation may be that the soybean proteins in SPI recovered from heat-denatured materials experienced special conformational changes during EE processing such that the emulsification performance was altered.

For the foaming properties, i.e., foaming speed and foaming capacity (Table 3), the differences were not significant. The differences for foam stability (*K* value) were minor, although they were statistically significant.

Overall, although the SPI and acid- and alcohol-washed SPC produced from EE meals had lower protein contents than their counterparts from white flakes, certain functional properties, such as emulsification capacity and dispersibility of acid-washed SPC, and emulsification capacity of SPI made from EE meals, were similar to, or higher than, those from white flakes. This indicates that certain soy protein products with good functional properties can be made from protein meals processed by extruding-expelling.

ACKNOWLEDGMENT

This research was financially supported by Iowa Soybean Promotion Board.

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[Received March 4, 2004; accepted June 14, 2004]