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Functional Properties of Soy Protein Isolates Prepared from Gas-Supported Screw-Pressed Soybean Meal

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Abstract White flakes (WFs) are obtained from dehulled flaked soybeans by extracting oil with hexane and flash- or downdraft-desolventizing the defatted flakes, and WFs are the normal feedstock used to produce soy protein ingredients. Gas-supported screw pressing (GSSP) is a new oilseed crushing technology in which traditional screw pressing is combined with injecting high-pressure CO_2, thereby producing hexane-free, low-fat, high-PDI soybean meal. The objectives of the present study were to evaluate yields, compositions, and functional properties of soy protein isolates (SPIs) produced from GSSP soybean meal and to compare these properties to those of SPIs produced from WFs. GSSP meals produced SPIs in significantly higher yields (59.7–63.1% vs. 51.6–61.1%), with greater free (0.05–0.40%) and bound fat (3.70–4.92%) contents than did WFs. There were no significant differences in protein contents of the SPI; all exceeded 90% protein content (db). SPIs prepared from GSSP meals had similar or slightly lower water-solubilities compared to SPIs prepared from WFs. SPIs prepared from GSSP meals had higher water-holding capacities and viscosities, and significantly better emulsifying and fat-binding properties compared to SPIs prepared from WFs. SPIs prepared from WFs had significantly better foaming properties compared to SPIs prepared from GSSP meals, which were attributed to the lower fat contents of SPIs prepared from WFs.

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Introduction

Soy protein isolate (SPI) is generally produced from solvent-extracted (defatted) soybean flakes or flour (DSF). Hexane is the current solvent of choice used to extract crude oil, and the (defatted) flakes are desolventized by means of flash- or downdraft-desolventizing to minimize protein denaturation [1]. These desolventizing methods are used to produce partially defatted soybean flakes known in the industry as white flakes (WFs), which undergo little protein denaturation and possess high protein dispersability index (PDI). High-PDI WFs are needed to obtain good protein extraction and high yields of SPI; however, concerns have been expressed over cost, availability, flammability [2, 3], and polluting and potentially toxic aspects of hexane [4]. To date, only hot screw pressing and extruding-expelling (EE) have gained commercial acceptance as alternative processes, but these processes cause extensive protein denaturation thereby reducing SPI yield [5].

Despite engineering challenges in making supercritical CO_2 (SC-CO_2) a continuous process, SC-CO_2 has long been promoted as a means of extracting oil from soybeans to produce DSF. SC-CO_2 leaves very little residual CO_2 in the oil or meal and CO_2 is nonflammable, nontoxic [4] and economical [6]. SC-CO_2 extraction produces DSFs with higher PDIs and less off-flavor in comparison to solvent-extracted DSF [6]; but, the high capital cost associated with SC-CO_2 has prevented adoption by the soybean processing industry. A new gas-supported screw press (GSSP) process developed by Crown Iron Works (Minneapolis, MN, USA) injects CO_2 under high pressure into a screw press to act as

a cooling and oil-displacement fluid thereby producing a unique soybean meal with high PDI and low residual fat content. The use of CO_2 as an extraction aid in the new GSSP process may provide similar advantages as is achieved with SC-CO_2.

SPI contains ~90% protein (db, dry basis) making it an excellent source of protein for use as food ingredients. The functional properties of SPIs determine their usage in food, and functional properties of SPIs are affected by the process used to produce them [5, 7]. Thus, it is important to determine the functional properties of SPIs produced from GSSP meals in order to determine the market potential for these new ingredients. The objectives of our study were to determine the yields, compositions and functional properties of SPIs produced from GSSP soybean meals and compare them to SPIs produced from soybean WFs. We hypothesized that GSSP soybean meal can be used to produce high quality SPI and that these products have similar or better functional properties than SPIs prepared from WFs. GSSP may be an ideal processing method to produce DSF for SPI manufacture from identity-preserved soybeans having specialty traits or being produced by value-added production methods.

Experimental Procedures

Materials

Two sources of soybeans were used in the present study: (1) conventionally grown commodity soybeans and (2) identity-preserved organically grown soybeans. Each soybean source was extracted by two different methods: (1) hexane extraction and (2) GSSP. The commodity hexane-extracted, downdraft-desolventized WFs (CDDWFs) were produced in the pilot plant of Crown Iron Works using a Model 2 shallow-bed extractor. Organic hexane-extracted air-desolventized WFs (OADWFs) were extracted in the pilot plant of the Center for Crops Utilization Research (Iowa State University) by using a French Oil Machinery Co. (Piqua, OH, USA) extractor-simulator. The GSSP meals produced from commodity soybeans (CGSSP) and organic soybeans (OGSSP) were processed and supplied by Crown Iron Works. The beans were dehulled using the Crown Iron Works hot-dehulling system, flaked and screw pressed using the Crown Iron Works Hyplex® screw-pressing process, which uses CO_2 to displace oil during screw pressing.

Upon receipt, all partially defatted meals were milled into soy flour (DSF) by using a Krups grinder (distributo federal, Mexico) until 100% of the material passed through a 50-mesh screen. Small quantities (~10 g) were milled at any one time to avoid heating and preserve the native protein state. The DSFs were stored in sealed containers and kept at 4 °C until used. The compositions of the GSSP meals and WFs are shown in Table 1.

SPI Production

As shown in Fig. 1, SPIs were prepared in the laboratory according to the methods of Deak and Johnson [8]. All SPIs were freeze-dried. SPIs were prepared in triplicate using 100 g of DSF from each of the four treatments.

Proximate Analyses and Mass Balance

The nitrogen contents of all samples were measured by using the Dumas combustion method [9] with a Rapid NIII Analyzer (Elmentar Americas, Inc., Mt Laurel, NJ, USA).

Table 1 Compositions of partially defatted soybean flours used to produce SPIs[a]

Compositional properties	Commodity		Organic	
	WF (downdraft desolventized)	GSSP	WF (air-desolventized)	GSSP
Protein (%, db)	54.8	53.2	57.9	55.2
Free fat (%, db)	1.6	6.5	0.7	4.5
Total fat (%, db)	2.5	6.9	2.6	6.1
PDI	81.7	68.4	91.7	57.7

[a] *WF* denotes white flakes and *GSSP* gas-supported screw pressing

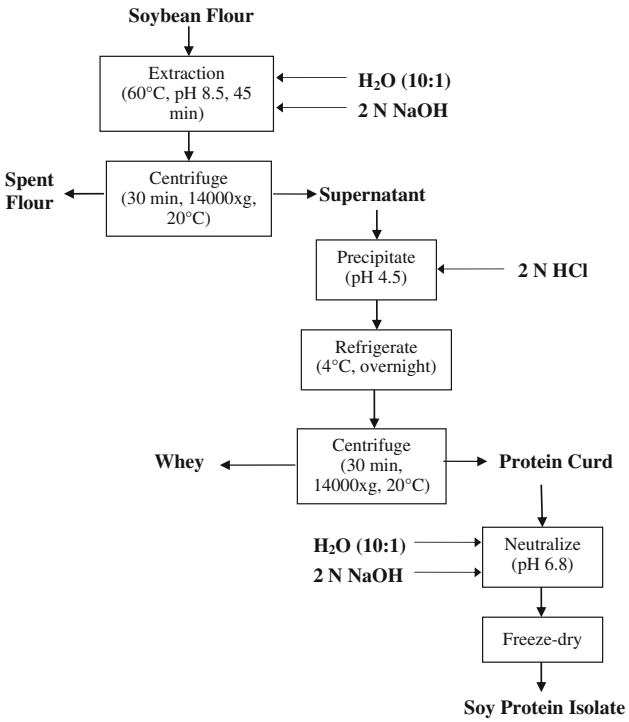

Fig. 1 Soy protein isolation procedure

These values were converted to Kjeldhal nitrogen concentrations using the conversion formula of Jung et al. [10]. The 6.25 × Kjeldahl N conversion factor was used to convert percentage of nitrogen to protein content. PDI was determined by N-PAL (St. Louis, MO, USA). Mass balances of protein and solids were determined for all treatments and yields were determined for all products. Crude free fat contents were determined by using the Goldfisch extraction procedure [11]. Total fat (free plus bound lipid) was determined by using the Mojonnier acid hydrolysis method [9]. Each sample was analyzed in triplicate and means reported.

Protein Compositions

Urea-SDS-PAGE gel electrophoresis was used to quantify individual protein components by using the methods of Wu et al. [12]. Lipoxygenase and soybean storage protein bands were identified by using a pre-stained SDS-PAGE MW standard, low range (Bio-Rad Laboratories, Hercules, CA, USA). Glycinin and β-conglycinin subunit bands were confirmed by using purified standards produced according to methods of O'Keefe et al. [13]. The amounts of all unidentified bands were summed and reported as "others". Densitometry was carried out by using Kodak one-dimensional (1D) Image Analysis, version 3.5 (Kodak, Rochester, NY, USA) on scanned images produced with a Biotech image scanner (Amersham Pharmacia, Piscataway, NJ, USA). SDS-PAGE results were calculated as percentage composition where total storage protein in a given fraction = [(sum of storage protein subunit bands)/(sum of all bands)] × 100. All measurements were replicated at least four times and the means reported.

Functionality

Thermal behavior, solubility, foaming, and emulsification properties were determined by using the methods of Deak and Johnson [14]. Dynamic viscosity was determined using the method of Rickert et al. [15]. Water-holding capacities (WHC) and fat-binding capacities (FBC) of the samples were determined by using the methods of Heywood et al. [16]. For all tests, the sample pH was adjusted to 7 by using either 2 N HCl or NaOH. Each sample was analyzed at least three times and means reported.

Statistical Analysis

The data were analyzed by Analysis of Variance (ANOVA). 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, USA).

Results and Discussion

Yields and Compositions

The four DSFs had similar protein contents (52–58%) but the GSSP meals contained significantly more fat (4.5–6.1% vs. 0.7–1.6%) than WFs (Table 1). Total fat contents were significantly higher than crude free fat contents. The amounts of dispersible protein (PDI, Table 1) were lower in GSSP meal than in WFs; however, it should be noted that air-desolventization represents the highest possible PDI and is not achievable in commercial practice as is downdraft desolventization. PDI values of ~80 are typical for commercial WFs used in SPI manufacture. GSSP meals were 12–24 points lower than for WFs (82) but were still reasonably high (58–68). Subsequent to the present study as high as 91.8 PDI with 6.3% fat (db) has been achieved. Typical screw-pressed and extruded-expelled soybean meals have PDIs in the range of 10 and 18, respectively [17]. Considering extruded-expelled soybean meal (typically containing 7–9% fat) is used in some instances for commercial SPI manufacture, GSSP offers considerable advantages.

Despite the residual fat contents in the GSSP meals being much higher than for WFs; the SPIs prepared from GSSP meal contained very low but slightly higher free fat contents than SPIs prepared from WFs (0.4% vs. 0.12%) (Table 2). Total fat contents were twice as high in SPIs produced from GSSP meals than in SPIs prepared from WFs (3.7–4.9% vs. 1.8–2.4%, respectively). The higher fat contents in SPIs prepared from GSSP meal only very slightly reduced protein contents; all SPIs exceeded 90% protein content, which is an important specification to meet. From our previous studies we found that commercial SPIs have free fat contents ranging from 0.12 to 0.74% and total fat contents ranging from 0.60 to 3.67%.

The yields of solids and protein as SPI from GSSP meals were significantly higher than for SPIs from WFs despite the GSSP meals having lower PDIs. This was surprising because PDI has been regarded by the industry as a good predictor for SPI yields from WFs, with higher yields from WFs having higher PDIs. The relationship between PDI and SPI yields may be different for GSSP meal than for WFs because the shear and amounts of moisture present and heat exposure during oil extraction are different. Protein denaturation may be different in GSSP meal than in WFs (additional evidence provided later). The solids and protein yields were also higher for the organic soybeans than for the commodity beans, perhaps indicating greater care taken during identity-preserved storage of the organic beans or differences in soybean variety.

Table 2 Yields and protein and fat contents of SPI[a]

Defatted soy flour used to prepare SPI	Solids yield (%)	Protein yield (%)	Protein content (%)	Free fat content (%)	Total fat content (%)
CDDWF	30.4[c]	51.6[c]	92.9[a]	0.12[b]	2.40[c]
CGSSP	34.6[b]	59.7[b]	91.8[b]	0.40[a]	4.92[a]
OADWF	38.0[a]	61.1[b]	93.2[a]	nd	1.82[d]
OGSSP	36.8[a,b]	63.1[a]	94.6[a]	0.05[b]	3.70[b]

[a] $n = 3$

Means within a column for a specific sample followed by different superscripts are significantly different at $p < 0.05$. *CDDWF* denotes hexane-extracted, downdraft-desolventized white flakes from commodity soybeans, *CGSSP* gas-supported screw-pressed commodity soybeans, *OADWF* hexane-extracted, air-desolventized white flakes from organic soybeans, *OGSSP* gas-supported screw-pressed organic soybeans and *nd* none detected

Table 3 Individual protein compositions of SPI (% of total protein)[a]

Defatted soy flour used to prepare SPI	Lipoxygenase	β-Conglycinin	Glycinin	Others
CDDWF	6[a]	45[b]	42[ab]	8[b]
CGSSP	nd	47[ab]	43[a]	10[a]
OADWF	4[a]	49[a]	39[b]	8[b]
OGSSP	nd	48[a]	41[ab]	11[a]

[a] $n = 3$

Means within a column for a specific sample followed by different superscripts are significantly different at $p < 0.05$. *CDDWF* denotes hexane-extracted, downdraft-desolventized white flakes from commodity soybeans, *CGSSP* gas-supported screw-pressed commodity soybeans, *OADWF* hexane-extracted, air-desolventized white flakes from organic soybeans, *OGSSP* gas-supported screw-pressed organic soybeans, and *nd* non-detectable

Compositions of Individual Proteins

The SPIs prepared from GSSP meals contained no lipoxygenase whereas the SPIs prepared from WFs did (Table 3). We did not wash the SPIs with water as is sometimes done in commercial practice, which would reduce the contents of highly soluble lipoxygenase. Since neither SPI was water washed, water washing does not account for the differences observed. The only apparent explanation is the different methods employed for fat extraction; however, we cannot offer plausible hypotheses at this time that would explain why one SPI contains lipoxygenase and the other does not. This phenomenon was observed in both laboratory and pilot plant trials (unpublished data). There were no significant differences in the glycinin and β-conglycinin composition of the WF and GSSP SPIs.

Thermal Behavior of SPI

In order to better understand why higher yields of solids and protein were achieved with GSSP meals despite having lower PDI, we examined the thermal properties of the proteins contained in the SPIs (Table 4). The shear, temperature, extraction time, pH, and other factors are different in laboratory and pilot-plant SPI processing versus laboratory PDI testing. The glycinin denaturation enthalpies for SPIs prepared from GSSP meals were higher than for the glycinin in SPIs prepared from WFs. The enthalpies for β-conglycinin were either the same or slightly higher in SPIs prepared from GSSP meal than in SPIs prepared from WFs. These findings indicate that the glycinin in GSSP meal was not as denatured as the PDIs suggested or the denaturation is different or something other than protein denaturation reduces PDI in GSSP meal. There were no practically significant differences in peak onset, off set and peak temperatures between SPIs produced by different fat-extraction methods. Therefore, we do not regard PDI to always be a good predictor of SPI yield from GSSP meal.

Solubility Profile

Protein solubility is the most important functional property for SPI because it affects most other functional properties and it is important for getting the protein incorporated into food. The solubility of a protein is affected by many factors including its processing and exposure to heat. The more denatured the protein, the lower its solubility [7]. The SPIs prepared from GSSP meals where either similar to or lower in solubility than SPIs prepared from WFs (Table 5). This suggests that solvent extraction caused proteins to unfold but not aggregate, thereby increasing solubility [5]. The PDIs of both WFs were higher than the PDIs of GSSP meals and may contribute to the higher solubilities of SPIs prepared from them. L'hocine et al. [5] also found that the defatting process used did not greatly affect the solubility of the SPI prepared from the meals.

Water-Holding Capacity (WHC)

WHC is defined as the ability of the protein to hold water against gravity [7]. SPIs prepared from GSSP meals held

Table 4 Thermal behaviors of SPIs[a]

Defatted soy flour used to prepare SPI	β-Conglycinin				Glycinin			
	On set Td (°C)	Off set Td (°C)	Peak Td (°C)	Enthalpy (mJ/mg protein)	On set Td (°C)	Off set Td (°C)	Peak Td (°C)	Enthalpy (mJ/mg protein)
CDDWF	66.4[a]	82.4[a]	73.9[b]	2.08[b]	85.1[a]	100.9[a]	93.1[a]	6.53[b]
CGSSP	69.1[a]	81.1[a]	74.4[b]	2.31[a]	83.8[a]	98.9[a]	90.2[b]	7.17[a]
OADWF	70.2[a]	83.4[a]	75.8[a]	2.32[a]	84.9[a]	102.9[a]	92.7[a]	6.45[b]
OGSSP	68.8[a]	81.6[a]	75.6[a]	2.17[ab]	84.7[a]	102.6[a]	93.3[a]	7.32[a]

[a] $n = 3$

Means within a column for a specific sample followed by different superscripts are significantly different at $p < 0.05$. *CDDWF* denotes hexane-extracted, downdraft-desolventized white flakes from commodity soybeans, *CGSSP* gas-supported screw-pressed commodity soybeans, *OADWF* hexane-extracted, air-desolventized white flakes from organic soybeans, and *OGSSP* gas-supported screw-pressed organic soybeans

Table 5 Solubilities, water-holding capacities and dynamic viscosities of SPIs[a]

Defatted soy flour used to prepare SPI	Solubility (%)	Water-holding capacity (g water/g sample)	Flow consistency index (*K*, mPa s)	Flow behavior index (*n*, dimensionless)
CDDWF	89.5[b]	1.2[d]	0.190[c]	0.669[a]
CGSSP	82.8[c]	3.5[b]	0.356[b]	0.596[b]
OADWF	91.8[a]	2.4[c]	0.256[c]	0.638[a,b]
OGSSP	88.6[b]	4.4[a]	0.616[a]	0.511[c]

[a] $n = 3$

Means within a column for a specific sample followed by different superscripts are significantly different at $p < 0.05$. *CDDWF* denotes hexane-extracted, downdraft-desolventized white flakes from commodity soybeans, *CGSSP* gas-supported screw-pressed commodity soybeans, *OADWF* hexane-extracted, air-desolventized white flakes from organic soybeans, and *OGSSP* gas-supported screw-pressed organic soybeans

significantly more water than the SPIs from WFs (Table 5). The SPIs prepared from organic soybeans, regardless of oil extraction method, held more water than the SPIs prepared from commodity beans. The WHCs of 2.4–4.4 g water/g protein for SPIs prepared from GSSP meals were similar to the WHCs for EE-processed soy flours (3.7–4.1 g water/g protein) [16]. WHC was not reduced as a result of the high fat content in the GSSP SPIs as was expected.

Dynamic Viscosity

Kinsella et al. [7] asserted that the shape of the protein molecule is a factor determining viscosity, and the shapes of the proteins are influenced by the processing treatment. Conformational changes in proteins, such as unfolding caused by alkali and heat treatment, can affect their viscosities. When using the Power Law model to describe dynamic viscosity, flow consistency index (K) measures resistance to flow (apparent viscosity); the greater the k value, the more viscous the dispersion. The flow behavior index or (n) measures how close to a Newtonian fluid the dispersion is; the closer the value to 1, the dispersion is more like a Newtonian fluid (such as water); $n < 1$ indicates shear thinning; and $n > 1$ indicates shear thickening.

The SPIs prepared from GSSP meals had significantly higher flow consistency indexes and lower or similar flow behavior indexes compared to the SPIs prepared from WFs, indicating SPIs prepared from GSSP meals have higher viscosities than SPIs prepared from WFs (Table 5). SPIs with higher solubility had lower viscosity as has been observed by Petruccelli et al. [18] who also found viscosity decreased as solubility increased. The desirability of high or low viscosity depends on the application the SPI is used in (some applications, such as wood adhesives, need high solids loading with low viscosity; others, such as food thickeners, need high viscosity). Deak et al. [14] showed that lower viscosity with similar protein contents may be due to less denaturation of glycinin and similar or more denaturation of β-conglycinin. The solvent-extraction and desolventizing process in making WFs probably causes β-conglycinin to dissociate into its subunits [19] and thus decrease viscosity. SPIs prepared from GSSP meals have more native β-conglycinin structure than SPIs prepared from WFs indicating less denaturation during the defatting process. This explains why the WFs had lower viscosities even though their protein contents were similar to or higher than those of SPIs prepared from GSSP meals. The organic soybeans produced SPIs with higher flow consistency

Table 6 Emulsification and fat-binding properties of SPIs[a]

Defatted soy flour used to prepare SPI	Emulsification capacity (g oil/g sample)	Emulsification activity (A% at 500 nm)	Emulsification stability index (dimensionless)	Fat-binding capacity (g fat/g sample)
CDDWF	510[d]	34.7[c]	142[b]	3.3[b]
CGSSP	678[b]	44.5[a]	279[a]	3.6[a]
OADWF	607[c]	38.1[b]	154[b]	3.0[b]
OGSSP	743[a]	47.0[a]	277[a]	3.7[a]

[a] $n = 3$

Means within a column for a specific sample followed by different superscripts are significantly different at $p < 0.05$. *CDDWF* denotes hexane-extracted, downdraft-desolventized white flakes from commodity soybeans, *CGSSP* gas-supported screw-pressed commodity soybeans, *OADWF* hexane-extracted, air-desolventized white flakes from organic soybeans, and *OGSSP* gas-supported screw-pressed organic soybeans

index than did commodity beans, suggesting differences due to soybean variety or storage history.

Emulsification Properties

Blending a protein into a lipid and water mixture causes the protein to unfold. Protein unfolding exposes hydrophobic regions to lipids and hydrophilic regions to water, thus reducing the surface tension between water and oil, and increasing emulsification capacity (EC) [14]. This ability is dependent on the structure and flexibility of the protein. SPIs prepared from GSSP meals exhibited significantly higher ECs, emulsification activities (EA) and emulsification stability indexes (ESI) than SPIs from WFs (Table 5). L'hocine et al. [5] also showed that the defatting process affects emulsification properties. The defatting process affects the amount of residual oil remaining; this could affect the hydrophobicity of the sample and thus affect emulsification properties.

EC is influenced by the protein content and PDI [16]. The SPIs prepared from GSSP meals had lower PDIs than the SPIs prepared from WFs, but they contained more residual fat, thus supporting our speculation that higher ECs for SPIs prepared from GSSP meals was due to the presence of phospholipids. Similar results were reported by Heywood et al. [16]. SPIs prepared from GSSP meals also had significantly better EAIs and ESIs than did SPIs prepared from WFs. This stability can be attributed to more native β-conglycinin in the SPIs prepared from GSSP meals. Rickert et al. [12] reported that β-conglycinin fractions have better EAs and ESIs compared to glycinin fractions.

Fat-Binding Capacity (FBC)

The amount of fat that soy protein can bind plays a very important role in foods. Soy protein is good at binding free fat, which is especially important in meat products. The SPIs prepared from GSSP meals bound significantly more fat than did SPIs prepared from WFs (Table 6). We attribute this to the differences in the way protein denatures in GSSP versus WFs as has been previously discussed.

Table 7 Foaming properties of SPIs[a]

Defatted soy flour used to prepare SPI	Foaming capacity (mL/mL)	Rate of foaming (mL/min)	Foaming stability ($1/K$ = mL min)
CDDWF	1.04[a]	5.7[b]	14.2[b]
CGSSP	0.96[b]	2.9[c]	8.3[c]
OADWF	1.12[a]	14.4[a]	59.1[a]
OGSSP	0.99[b]	4.6[b]	11.3[c]

[a] n = 3

Means within a column for a specific sample followed by different superscripts are significantly different at $p < 0.05$. *CDDWF* denotes hexane-extracted, downdraft-desolventized white flakes from commodity soybeans, *CGSSP* gas-supported screw-pressed commodity soybeans, *OADWF* hexane-extracted, air-desolventized white flakes from organic soybeans, and *OGSSP* gas-supported screw-pressed organic soybeans

Foaming Properties

The SPIs prepared from GSSP meals had lower foaming capacities and slower foaming rates than SPIs prepared from WFs (Table 7). Fat depresses foaming capacity (FC) and SPI prepared from GSSP meal contains more free and bound fat [20]. The additional protein-bound fat makes the protein less mobile and less interactive with the hydrophobic interface of air cells. There were no differences in FCs between organic versus commodity beans, but the organic soybeans produced SPIs with faster rates of foaming than did SPIs produced from commodity beans. Foaming stability (FS) is expressed as $1/k$, therefore the higher the FS value the more stable the foam. SPIs prepared from GSSP meals also formed the least stable foams than did SPIs prepared from WFs. Rickert et al. [12] found that denatured proteins unfold without much resistance at the air water interface, therefore forming better foams.

Conclusions

The new GSSP technology produced SPI with unique properties. The process can be used as an alternative to solvent oil extraction from soybeans and enables production of organic SPI. GSSP SPIs all had greater yields with higher fat contents than SPI prepared from WFs. GSSP SPIs contained >90% protein. Important functional properties of GSSP SPI include better WHC, higher viscosity, and better emulsification and fat-binding properties than SPI prepared from WFs, but similar to slightly lower water-solubility and foaming properties. Our study indicates that the oil extraction method used can affect the functional properties of SPI. GSSP is an important new technology for crushing soybeans and is capable of producing new soy protein ingredients with unique compositional and functional properties.

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