Compositional, functional and sensory properties of protein ingredients

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Compositional, functional and sensory properties of protein ingredients prepared from gas-supported screw-pressed soybean meal

by

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# TABLE OF CONTENTS

ABSTRACT

CHAPTER 1. GENERAL INFORMATION
    Introduction 1
    Thesis Organization 2
    Literature Review 3
    References 25

CHAPTER 2. FUNCTIONAL PROPERTIES OF SOY PROTEIN ISOLATES PREPARED FROM GAS-SUPPORTED SCREW-PRESSED SOYBEAN MEAL 34
    Abstract 34
    Introduction 35
    Experimental Procedures 36
    Results and Discussion 40
    Conclusions 49

CHAPTER 3. FUNCTIONAL PROPERTIES OF JET-COOKED AND HYDROGEN-PEROXIDE-TREATED SOY PROTEIN ISOLATES 52
    Abstract 52
    Introduction 53
    Experimental Procedures 54
    Results and Discussion 57
    Conclusions 67

CHAPTER 4. SENSORY PROPERTIES OF SOY PROTEIN ISOLATE AND GLYCININ-RICH AND β-CONGLYCININ-RICH FRACTIONS PREPARED FROM GAS-SUPPORTED SCREW-PRESSED SOYBEAN MEAL 70
    Abstract 70
    Introduction 71
    Experimental Procedures 73
    Results and Discussion 78
    Conclusions 85

CHAPTER 5. GENERAL CONCLUSIONS
    General Discussion 89
    Recommendations for Future Research 91
    Acknowledgements 92

APPENDIX A. SENSORY PANEL QUESTIONNAIRE 93

APPENDIX B. BASIC SENSORY TESTING 94

APPENDIX C. SENSORY SCORE SHEET FOR SOY PROTEIN SAMPLES 96
Soy protein products are gaining importance as ingredients in the food industry. A number of soybean meals have been investigated as starting materials for the production of soy protein ingredients. Hexane-extracted and flash-desolventized soybean meals, known as white flakes (WF), are most commonly used, but have disadvantages of containing solvent residue and being too expensive for processing identity-preserved soybeans. Gas-supported screw pressing (GSSP) is a new soybean oil-extraction process that combines screw pressing with injecting carbon dioxide (CO₂) under pressure. The objective of the present research was to investigate GSSP meal and its protein products by determining yields, composition, functional properties, preservation methods and sensory properties. The properties of GSSP meal proteins were compared to traditional soy protein products produced from WF.

For the laboratory-scale study, analytical, chemical and functionality tests were performed on the starting materials and isolated soy proteins. Soy protein isolate (SPI) prepared from GSSP meal had higher protein yield, fat content, water-holding capacity (WHC) and viscosity, and better emulsification and fat-binding properties than SPIs prepared from WF.

The SPIs produced in the pilot plant were analyzed for composition and functionality. Hydrogen peroxide (H₂O₂) treated SPIs were compared to jet-cooked SPIs. GSSP SPIs did not differ in functionality from SPIs prepared from WF, except for having lower solubility and poorer foaming properties. H₂O₂ used as a preservative improved solubility, emulsification and foaming properties and reduced glycinin and β-conglycinin (β-con) denaturation.

A descriptive sensory panel study with 12 trained panelists evaluated the aroma, flavor and mouthfeel of SPI and glycinin-rich (gly-rich) and β-conglycinin-rich (β-con-rich) soy protein fractions extracted from both GSSP meal and WF. Protein products prepared
from GSSP meal were similar to protein products prepared from WF except for having greater mouthcoating. Regardless of starting material, the gly-rich and β-con-rich fractions had stronger fishy aroma, less floury aroma, less raw beany aroma and less floury flavor than the SPIs. Hunter color LAB data indicated GSSP meal was more yellow (higher b* value) in color compared to WF. SPIs were darker (lower L* value) than than the gly-rich and β-con-rich fractions.

Overall, protein products prepared from GSSP meal were similar in composition, functional and sensory properties to protein products prepared from WF. These findings demonstrate that the GSPP process can produce defatted meals suitable for manufacturing soy protein ingredients. Because GSPP plants can be profitable at low capacity (50 mt/day) compared to solvent extraction (3000 mt/day), GSPP is suitable for processing identity-preserved soybeans that contain value-added traits. Additional benefits are that there are no concerns over residual organic solvents and the process complies with “organic” definitions.
CHAPTER 1. GENERAL INFORMATION

Introduction

The United States produced an estimated 2,973 million bushels of soybeans in the 2008 crop year (U.S. Department of Agriculture, 2009). Approximately 10% of the soybean crop is used directly for human consumption. An estimated 4 to 5% of the total soybean meal is processed into soy protein ingredients (soy flour, soy protein isolate and soy protein concentrate). Although it seems like a small amount, soy protein food ingredients are gaining wide acceptance in the United States. Soy protein is a good quality protein with the highest protein digestibility corrected amino acid score (PDCAAS) among the vegetable proteins. Today, soy protein ingredients are also gaining popularity because recent research indicates soy protein has health benefits.

Traditional hexane-extracted and flash-desolventized soybean meal, also known as white flakes (WF), is used in the production of soy protein isolate (SPI). The preferred solvent used for oilseeds extraction is hexane, which is not only flammable, but can be toxic and expensive (Johnson 1998, Friedrich and List 1982, Li et al. 2006). Traditional solvent extraction does not enable the production of “organic” soy protein ingredients because of the large scale required to be cost effective. Hence, researchers have been developing alternative oil-extraction processes for identity-preserved processing and determining the compositional and functional properties of the high-protein soybean meals obtained by using these processes.

Not only is the safety of protein ingredients important, they also need to have good compositional and functional properties so they can be incorporated into different food systems. Flavor is a critically important attribute when using soy protein ingredients in foods. Consumers associate undesirable beany off-flavor with soy protein products.
(Rackis et al. 1979). Hence, studying the sensory attributes of any new soy ingredient product is not only resourceful but necessary.

The present research investigates the potential for a new gas-supported screw-pressing (GSSP) process for extracting oil to produce meal from which highly functional soy protein products can be produced. In this process, oil is extracted from dehulled and flaked soybeans by injecting liquefied CO2 under pressure into a screw press, producing a soybean meal free of solvent residue that can be used to produce functional soy proteins. When the CO2 flashes (changes from a liquid to a gas absorbing energy) as it exits the screw press, the temperature is immediately reduced. Because of short exposure time to high temperatures at low moisture, little protein denaturation occurs and the protein remains highly soluble. High solubility protein is needed to extract soy protein in high yield when making SPI or fractionated soy protein ingredients.

Our hypothesis was that GSSP soybean meal can be used to produce high-quality SPI and fractionated soy protein ingredients with similar or better properties than protein products prepared from WF. The objectives the present studies were: 1) to determine the yields and compositional and functional properties of SPIs produced from GSSP meal and WF in laboratory SPI simulation (proof of concept); 2) to evaluate the effects of oil-extraction and preservation methods on the yields and compositional and functional properties of SPIs prepared from GSSP meal and WF in the pilot plant (scale-up); and 3) to evaluate the sensory properties of SPI and glycinin-rich and β-conglycinin-rich fractions produced from GSSP meal (sensory study).

**Thesis Organization**

This thesis consists of five chapters and three Appendixes. Chapter 1 includes a general introduction and a literature review. The ensuing chapters 2, 3 and 4 are journal manuscripts to be published in the *Journal of American Oil Chemists Society*. Chapter 2 entitled “Functional properties of soy protein isolates prepared from gas-supported screw-
pressed soybean meal” has been publication. Chapter 3 entitled “Functional properties of jet-cooked and hydrogen-peroxide-treated soy protein isolates” and chapter 4 entitled titled “Descriptive sensory analysis of soy protein isolate and glycinin-rich and β-conglycinin-rich fractions prepared from gas-supported screw-pressed soybean meal” will also be submitted for publication. Chapter 5 includes a general discussion summarizing pertinent findings and recommendations for future research based on the findings of the present research. The Appendixes include relevant but not publishable information and not included in the preceding chapters.

**Literature Review**

**Soybeans**

The soybean plant is native to southeastern Asia, where it was used for its medicinal properties and high protein content (Johnson et al. 1992). Since the 20th century, demand for soybean oil and protein from defatted meal have substantially increased. Soybean meal is widely used for supplementing protein in animal feeds. Soybeans rank highest among all food crops for its protein content and second among all legumes for its oil content (Liu 1999). Since the 1950’s, production of soy protein products for human consumption has increased; the United States alone produces more than 454 million kg per year of soy products for human consumption (Endres 2001). Protein products produced from soybeans include soy flakes, flour, protein concentrates, SPI, texturized soy proteins and spun proteins. These ingredients are used in the production of foods such as baked foods, dairy, meat, breakfast cereal, infant formula, as well as dairy and meat analogs (Lusas and Rhee 1995).
Health benefits

Soy protein products are excellent sources of high quality protein, are low in saturated fat, and contain dietary fiber and nutraceutical-valued isoflavones. Soy protein ingredients have been attributed a number of beneficial effects on human health such as lowering blood cholesterol levels, preventing obesity, providing nutrition and possibly even play a beneficial role in preventing diseases (cancer, osteoporosis, menopausal disorders and cardiovascular diseases) (Xiao 2007, Mateos-Aparicio et al. 2008, Takamatsu et al. 2003). A rat-feeding study showed that consuming a soy protein diet resulted in 40 to 47% of its iron being converted to hemoglobin iron (Pellett et al. 1990). Consumption of soy protein has a beneficial affect on renal function (Anderson 2007) and on reducing weight, adiposity (Cope et al. 2008) and incidence of breast cancer (Warri et al. 2008). SPIs contain from 88 to 164 mg/100 g of isoflavone (Genovese et al. 2007), which has also been reported to provide health benefits in humans (Xiao 2007, Isanga and Zhang 2008, Adlercreutz and Mazur 1997).

Soybeans, however, contain bioactive compounds that may have adverse health effects (Isanga and Zhang 2008). In addition, soybeans also contain digestive enzyme inhibitors, which lead to poor digestibility; but, this can be eliminated by proper heating (Friedman and Brandon 2001). Some other limitations to the widespread consumption of soybeans and its products are its allergenicity (Ballmer-Weber and Vieths 2008), beany taste and odors. A study on factors associated with consumers eating a healthy breakfast cereal determined consumers avoid soy-based products due to unfavorable taste despite widespread promotion of soy as a healthy ingredient (Lee et al. 2007).
**Composition**

Soybeans contain approximately 40% protein, 20% oil and 35% carbohydrates on dry basis (Perkins 1995). The majority of soybean proteins are storage proteins (65-80%) as opposed to functional or structural proteins. Soy storage proteins are composed of two primary proteins – glycinin (primarily 11S) and β-conglycinin (primarily 7S), and their contents vary with soybean variety and environmental conditions under which they are grown. Based on solubility, soy proteins they may be further classified as albumins (water soluble) and globulins (salt soluble). Most of the soy storage proteins are globulins and are deposited in protein bodies, which are spherical in shape and range in size from 2 to 20 μm (Snyder and Kwon 1987). Crop year and genotype differences in the soybeans affect the relative proportions of glycinin and β-conglycinin, and thus the functional and chemical properties of soy protein products (Khatib et al. 2002).

Electron microscopy has shown that soybeans also have lipid-containing spherosomes ranging in size from 0.2 to 0.5 μm between protein bodies (Saio and Watanabe 1968). The oil contents of 10 normal soybean genotypes grown in Arkansas were reported to range from 16.3 to 21.6% and genotype affected fatty acid composition (Liu et al. 1995).

Soybean flours contain approximately 17% soluble and 21% insoluble carbohydrates (Perkins 1995). Soybeans contain approximately 4.1% sucrose, 1.1% raffinose and 3.7% stachyose, which vary with genetics and environmental conditions (Vaidehi and Kadam 1989). Soybean flour obtained from soybeans high in sucrose and low in stachyose was similar in protein composition to flour from normal defatted soybeans (Deak et al. 2006a).
Major soy proteins

Glycinin

Glycinin makes up 25-35% of the total seed protein (Murphy and Resurreccion 1984) and is classified as a legumin. Glycinin is a hexamer (Fig. 1) of about 360 kDa and composed of 12 polypeptides – 6 acidic (34-44 kDa) and 6 basic (20 kDa). The polypeptides exist as acidic-basic pairs, linked by a single disulfide bond, often called “jelly rolls” because of its structure. These paired polypeptides then form two trimers of 6 polypeptides each, associated by hydrophobic and hydrogen bonds. The structures of the two trimers are visible through electron microscopy and are described as donuts, because they associate with each other to form the glycinin hexamer (Badley et al. 1975).

These glycinin subunits dissociate under extreme environmental conditions such as at extreme pH, ionic strength and heat. Two species of glycinin have been reported to exist, one dissociable at low ionic strength and the other non-dissociable (Utsumi et al. 1987). Glycinin denatures rapidly, starting at around 90°C. A number of studies have been done to identify the acidic-basic peptides, their genetics and composition (Nielsen 1985, Nielsen et al. 1989, Stastwick et al. 1981, Utsumi et al. 1997) to better understand the behavior of soy proteins in relation to its structure.
**β-Conglycinin**

β-Conglycinin is the other major storage protein comprising soy protein and is classified as a vicilin. It has a molecular mass of 125-170 kDa and is composed of three subunits $\alpha$, $\alpha'$ and $\beta$. Early reports erroneously suggested the presence of a 4th subunit $\gamma$ (Thanh and Shibasaki 1977). These subunits come together to form a trimer (Fig. 2). There are no disulfide bonds between the subunits; they associate by strong hydrophobic and hydrogen bonds. The trimer contains two cysteine residues, one in the $\alpha$ subunit and the other in the $\alpha'$ subunit. The trimer also contains five methionine residues, one in the $\alpha$ subunit and four in the $\alpha'$ subunit (Utsumi et al. 1997). All three subunits are N-
glycosylated, α and α’ contain additional extension regions (Thanh and Shibasaki 1976, Maruyama et al. 1998).

**Figure 2. Structure of β-conglycinin (from Maruyama 2001).**

The ribbon diagrams of the recombinant (A and B) and native (C and D) β homotrimers.

Maruyama et al. (1998) studied the roles of the glycans and extensions in the folding, assembly and structure of β-conglycinin. These regions play a role in establishing the dimensional structure of β-conglycinin but not density or thermal stability. They also suggest that the extension regions play a role in preventing aggregation. β-Conglycinin denatures slowly with increasing temperature starting at around 70°C.
**Soy protein isolation**

SPI is one of the protein-rich food ingredients derived from soybean meals. Scientists have been investigating the isolation of soy protein as early as 1903 (Johnson et al. 1992). Soy proteins are isolated on the basis of solubility at different pHs (Fig. 3). The traditional SPI process was described by Wolf in 1983. The basis steps include solubilizing the protein in WF produced by dehulling the beans, flaking, extracting the oil with hexane and desolventizing the protein-rich defatted meal by flash desolventizing to reduce protein denaturation. The proteins are extracted by solubilizing in water at 60°C, 10:1 solvent:solids ratio and pH 8-11, and removing the insoluble fiber by centrifuging. The protein is then precipitated by adjusting the pH to 4.2-4.5 and the protein curd is removed from the soluble sugars (whey) by centrifuging. The protein curd is water-washed and centrifuged again. This washed protein curd is neutralized to pH 6.8, and then spray-dried. SPIs are traditionally prepared from WF, but recently it has been shown that extruded-expelled soybean meal (Wang et al. 2004a) and gas-supported screw-pressed (GSSP) soybean meal (Deak et al. 2008) can be used.

Particle size distribution of the soy flour affects the yields of SPI obtained; the smaller the particle size, the higher the recovery of protein, whereas the purity (protein content) of the SPI is not affected by particle size (Russin et al. 2007). The temperature at which soy protein is extracted does not affect protein yields and solids. Extraction temperature and drying method, however, affect functional properties (Deak and Johnson 2007). Deogara et al. (1992) reported on the affects on the functional properties of SPIs that were heated at different temperatures during isoelectric precipitation.
Figure 3. Soy protein isolation procedure (adapted from Deak and Johnson 2007).

Fractionating soy storage proteins

Glycinin and β-conglycinin exhibit different functional properties and, hence, may have different uses. Wolf et al. (1962) was able to fractionate relatively pure glycinin by using cryoprecipitation and fractionation, but the yield was only 25%. He also investigated factors affecting the purity and yield of the glycinin fraction, including but not limited to pH, temperature and extraction ratio (Wolf et al. 1967).
Another study by Koshiyam (1965) reported on a procedure to fractionate glycinin and \(\beta\)-conglycinin. This procedure required a number of steps before relatively pure fractions could be obtained. Thanh and Shibasaki (1976, 1977) reported on a simpler procedure to fractionate glycinin and \(\beta\)-conglycinin; this procedure is considered the “gold standard” of laboratory soy protein fractionation. The procedure was based on solubility differences of each protein at different pHs.

O’Keefe et al. (1991) modified Thanh and Shibasaki’s procedure improving the purity of the \(\beta\)-conglycinin fraction, but with low yields. Nagano et al. (1992) modified Thanh and Shibasaki’s procedure and produced >90% pure fractions of glycinin and \(\beta\)-conglycinin. All the aforementioned procedures were developed for laboratory use and not for commercial production.

Wu et al. (1999a) were able to produce a glycinin-rich fraction, a \(\beta\)-conglycinin-rich fraction and an intermediate fraction (a protein mixture) in the pilot plant by modifying Nagano’s laboratory procedure. Their process produced fractions with similar purities to those obtained in the laboratory. In an effort to eliminate the intermediate fraction and improve the yields of the glycinin-rich and \(\beta\)-conglycinin-rich fractions, Wu et al. (2000) developed a simplified process that used pH adjustment and ultrafiltration to produce a glycinin-rich and a \(\beta\)-conglycinin-rich fractions with twice as much yield of the \(\beta\)-conglycinin fraction but with lower purity. Saito et al. (2001) used phytase to aid in the fractionation of \(\beta\)-conglycinin.

Rickert et al. (2004) improved the Wu-Nagano’s modified procedure and obtained higher \(\beta\)-conglycinin yields, but with low purity. Khorshid et al. (2007) were able to fractionate glycinin and \(\beta\)-conglycinin from soymeal using carbon dioxide at pressure of 30 bar, temperature of 21-23 °C and a pH range of 5.4-5.6. The Deak and Johnson procedure (Deak and Johnson 2005, Deak et al. 2006b) developed a simplified fractionation procedure using CaCl\(_2\) and NaHSO\(_3\) as the reducing agent. This procedure produced glycinin-rich and \(\beta\)-conglycinin-rich fractions with >80% purities (Deak et al. ...
2007) and is regarded to be the first commercially viable soy protein fractionation process.

**Electrophoresis**

Electrophoretic separation of soy protein using ion-exchange chromatography (Thanh and Shibasaki 1976) or SDS-polyacrylamide (SDS-PAGE) gel electrophoresis (PAGE) (Fontes et al. 1984) is commonly done to identify, separate and quantify soy proteins. Electrophoresis identifies the different protein components of SPI including lipoxygenase (Lx) (Iwabuchi and Yamauchi 1987), α, α', β and γ subunits of β-conglycinin (Thanh and Shibasaki 1977, Davies et al. 1985), AB (acidic-basic) subunits and A (acidic) and B (basic) subunits of glycinin polypeptides (Nielsen et al. 1985). SDS-PAGE gels can be successfully run on both reduced and native SPIs to identify the components listed above (Petruccelli and Anon 1995).
Thermal behavior

The thermal behavior of a protein relates to its functional properties and thus use in foods. Heating soy proteins above 70°C causes protein structures to unfold and subunits to denature and dissociate (Morr 1987). Thermal behavior is affected by a number of factors including pH, protein concentration and heat treatment.

Heat coagulation time of SPI proteins increases with increased pH (Rayan et al. 2008). Native soy proteins at alkaline pH (pH 9) are more stable than at acidic pH (pH 3.8) (Mohamed and Xu 2003). Similar results were obtained with glycinin, which denatured faster at lower pHs (Renkema et al. 1999). Glycinin in heat-treated SPI denatured faster at pH 11 than at pH 7; this change in denaturation rate was not observed with the β-conglycinin component when the pH was increased. It has been suggested that glycinin undergoes conformational changes and is 50% denatured at pH 11 (Petruccelli and Anon 1996).
In another study, thermal denaturation of β-conglycinin was affected by changes in pH and ionic strength by influencing the environment surrounding the protein, which has greater effects on its aggregation than heating (Iwabuchi et al. 1991). When heated at 100°C for 30 min, both glycinin and β-conglycinin completely denature. While heating at 80°C for 30 min causes β-conglycinin to completely denature, glycinin is more heat stable and is only partially denatured (Sorgentini et al. 1995).

**Functionality**

**Solubility profile**

Solubility is the most important functional property because it affects most other protein functionalities (Bian et al. 2003, Kinsella 1979). The solubility of a protein is affected by many factors including its processing history, especially exposure to heat. The solubility of protein decreases with increasing denaturation (Kinsella 1979). Solubility of SPI is affected by a number of factors, including pH, ionic strength and temperature. Soy proteins typically exhibit a U-shaped trend in solubility with respect to pH. Solubility is typically high at the extreme ends of the pH scale with little or no solubility around its isoelectric point (pI) of pH 4.5 (Wolf 1983, Kinsella 1979).

Temperature increase did not significantly affect protein solubility, except for a few SPIs that were reported to increase in solubility by 20% when temperature was increased to >50°C (Lee et al. 2003). Dias et al. (2003) investigated the solubility of the reduced acidic and basic subunits of glycinin in comparison to the subunits of glycinin. They reported that the acidic subunits are more soluble than those of the glycinin fraction, and the basic components are not soluble over pH 3 to 10.
**Water-holding capacity**

Water-holding capacity (WHC) is the ability of a protein matrix to hold water against gravity (Kinsella 1979). Denatured proteins in SPI have higher water-imbibing capacity than native proteins. This is attributed to the unfolding of the denatured protein, which exposes more water binding sites. In the presence of salts, the WHC of protein increases (by 50-100%), salts cause more protein-protein interaction, which results in the proteins aggregating and precipitating thereby binding more water (Jovanovich et al. 2003). Similar results were reported by Gonzalez et al. (2001), who observed protein isolates containing more denatured proteins had lower solubilities and higher water absorption capacities. WHC is not only affected by the state of protein denaturation, but also by the extent of denaturation and the type of protein aggregation. The two protein fractions (glycinin and β-conglycinin) exhibit different aggregation properties (Sorgentini et al. 1991).

The proportion of the two proteins (glycinin and β-conglycinin) present in soy protein ingredients affects WHC. Protein-protein interaction between these fractions is greatest when they are present in a molar ratio of ~1, which results in a low WHC. WHC decreased as the β-conglycinin-to-glycinin ratio increased (Yao et al. 1988). The presence of sodium chloride decreases WHC of proteins by preventing the protein’s polar amino acids from interacting with water (Yao et al. 1988).

**Dynamic viscosity**

Viscosity is the resistance of a protein in solution to flow and is measured when exposing the proteins in solution to continuous shearing at constant rate (Deak 2004). This is an important property for proteins when incorporated in foods like soups, beverages, batters and meats. Kinsella (1979) suggested that the shape of the protein is one of many factors determining its effect on viscosity, which may be influenced by
processing treatment. Conformational changes in proteins, such as unfolding caused by alkali and heat, can affect soy protein viscosity.

Wagner et al. (1992) evaluated the rheological properties of commercial SPIs and reported that moisture and protein concentrations are interdependent factors affecting rheological behavior. The addition of sodium chloride and sodium sulfite salts to SPI shields the protein from interacting with water, thereby, reducing WHC and increasing viscosity. Thermal treatments of SPI dispersions increases viscosity, with both partially and totally denatured proteins even with the addition of salts. This suggests increased protein-protein interaction as protein denatures.

Soy protein fractions (glycinin and β-conglycinin) exhibit similar behavior, viscosity increases with increased heat treatment (Bian et al. 2003). Yao et al. (1988) studied the effects of changes in the ratio of glycinin and β-conglycinin in soybeans during maturation on its rheological properties. SPI produced from mature seeds was more viscous than SPI produced from immature seeds. The lowest viscosity occurred when 35% β-conglycinin and 65% glycinin was present. Dias et al. (2003) reported that the basic subunit reduced by using sodium bisulfite, had the greatest viscosity. All the subunits had viscosities higher than that of intact native glycinin, except for the β-mercaptoethanol-reduced low-molecular weight acidic subunit (Dias et al. 2003).

Emulsification properties

Introducing protein to a lipid-water mixture causes native protein structures to unfold. The unfolding exposes hydrophobic regions of the protein to the lipid and hydrophyllic regions to the water, thus reducing the surface tension between the water and oil. This ability is dependent on its structure and flexibility (Kinsella 1979).

Emulsification capacity (EC) and stability (ES) of soy protein are lowest at the isoelectric points and increase at pHs below or above this point. Emulsification capacity
and stability are also higher for the β-conglycinin-rich protein fraction than for the glycinin-rich fraction over the pH range 2-10 (Aoki et al. 1980). Emulsification stability and activity have good linear correlation with the surface hydrophobicity of the β-conglycinin-rich fraction. The more hydrophobic the surface of the protein, the better the emulsification properties. The surface hydrophobicity of the β-conglycinin-rich fraction increases with heat denaturation (Kato et al. 1983). Bian et al. (2003) reported that EC of the β-conglycinin-rich fraction is higher than the glycinin-fraction when comparing two SPI extraction processes.

Dias et al. (2003) studied the emulsification behaviors of the individual subunits of glycinin and reported that the acidic subunit and basic subunit both have higher EC’s than the intact native glycinin, with the low-molecular-weight acidic subunit being the highest. The glycinin:β-conglycinin ratio affects the ability of the soy protein to emulsify. Considerable differences in this ratio have been observed among various soybean genotypes. Genotypes with the high glycinin:β-conglycinin ratios and low β-conglycinin concentrations have high emulsification activity index (EAI). It has also been reported that glycinin when in the monomeric form enhances emulsion stability and that the ratio of monomeric and dimeric glycinin is important for emulsion stability (Pesic et al. 2005). Emulsion stability index (ESI) is higher for soy protein fractions with isoelectric points between 5.6 and 5.1 than between 5.1 and 4.5 (Chove et al. 2001).

At higher protein concentrations (~1.25-1.5 mg/mL), SPI has better emulsion forming ability at pH 6 than at pH 7 (Santiago et al. 1998). Improved SPI emulsification properties are observed when pH is increased from 7 to 9. This was attributed to increased salt content when pH is changed or due to changes in degree of protein association-dissociation.

Exposing SPI to short thermal treatments improves emulsification properties. (Petruccelli and Anon 1996). Another study found heating improved emulsification properties of SPI compared to its native form; heating increased hydrophobicity.
Reducing disulfide bonds of SPI by chemical treatments with urea or guanidine hydrochloride also improved the emulsification properties of the SPI (Nir et al. 1994).

**Foaming properties**

In order to be able to create foam, the protein needs to unfold, adsorb to the air-water interface and reduce surface tension of water. Surface hydrophobicity is highly correlated with foaming power. There is no correlation between surface hydrophobicity with foaming stability, which might be affected by denaturation of the protein rather than surface hydrophobicity (Kato et al. 1983). Foaming properties decline as a result of decreased adsorption at pH 5, which is close to the isoelectric point of soy protein. Interfacial characteristics improve with increasing ionic strength, even at acidic pH. Close relationships exist between foaming capacity and diffusion of soy globulin to the air-water interface and between foaming stability and surface pressure. At pH 7, β-conglycinin has better foaming capacity and stability than glycinin (Ruiz-Henestrosa et al. 2007). Yu and Damodaran (1991) found foams prepared with β-conglycinin have lower foaming stability than foams prepared with SPI and glycinin. Changing the proportions of glycinin and β-conglycinin in SPI did not improve foaming properties (Petruccelli and Anon 1995).

**Soybean meal extraction**

Oil is extracted from soybeans leaving behind a protein-rich meal that is gaining importance for producing food ingredients for human consumption. The important properties for an extraction solvent are; high solvent power, nontoxicity, nonflammability, low specific heat, low heat of vaporization and low cost (Johnson 1998). Soybean meals obtained by using different oil extraction processes contain different amounts of neutral oil and may contain different amounts of polar lipids (Wu
and Wang 2003). The oil extraction process also affects its protein composition and structure, which results in differences in SPI functionality.

**Hexane extraction**

Hexane is the most commonly used solvent for soybean oil extraction. The resulting meals are known as white flakes (WF) when the solvent is evaporated by flash desolventization, vapor desolventization, or downdraft desolventization. Using hexane as a solvent is expensive and its availability can be uncertain. It is highly flammable and explosive when in contact with air and a source of ignition. Hexane is also not selective when extracting oil from soybeans; hence the extracted oil requires further refining adding cost (Friedrich and List 1982). Commercial hexane-extracted, toasted, defatted soy flour and SPI contained 90 to 410 μg/g and 6 μg/g residual hexane, respectively (Honig et al. 1979).

**Extruding-expelling**

Extruding-expelling (EE) has the advantage over solvent extraction because it requires low capital costs, simple machinery, no solvent, and can be used for small-scale identity-preserved processing. EE, however, causes extensive heat-denaturation of the proteins resulting in protein products with very poor functional properties. Hydrothermal cooking (jet cooking) of the extruded soybean meal has improved the functional properties of heat-denatured proteins by disrupting protein aggregates (Wang et al. 2004b).

SPI yields from EE soybean meals are lower than that obtained from WF. The yield of SPI from WF is proportional to a decrease in protein dispersibility index (PDI) or nitrogen solubility index (NSI) of the EE soybean meal. Heywood et al. (2002) investigated and reported on the functional properties of EE meals. The protein content of
SPI prepared from EE meal was about 80%, which was significantly lower than the standard (>90% db) for SPI prepared from WF. The SPI prepared from EE meal had similar or better functional properties than SPI prepared from WF (Wang et al. 2004a).

The residual oils and PDIs of EE meals range from 7.0 to 11.7% and 32 to 50, respectively. Crowe et al. (2001) studied the oil contents and PDIs of extruded meals when varying extrusion conditions and reported the residual oil contents of extruded meals could range from 4.7 to 12.7% and the PDIs from 12.5 to 69.1. Meals extruded at lower temperatures achieved higher PDIs than the meals with higher residual oil contents. In meals extruded at temperatures of <117°C, lipoxygenase was present.

**Screw-pressing**

The screw-pressing process is similar to extruding-expelling except the extruding step is replaced with cooking in a stack cooker or a rotary-tube dryer before the soybean is put into the press, resulting in extensive heat denaturation. Wang and Johnson (2001b) evaluated extruded-expelled and screw-pressed meals and reported that the screw-pressed meals had higher oil contents and lower protein and moisture contents, and lower PDIs compared to solvent-extracted meals.

**Alcohol extraction**

L’Hocine et al. (2006) showed that ethanol extraction and aqueous extraction can be used as alternatives to hexane extraction. The SPIs produced from these meals had protein contents of 90 and 84%, respectively, and their functional properties were similar to those of SPIs prepared from WF. Decreased emulsification activity was observed with the SPIs prepared from soybean meals prepared by alternative means to hexane extraction. Improved emulsion stability and foaming properties were observed with
aqueous-extracted SPIs. Decreased fat-holding capacity was observed with SPIs prepared from methanol-extracted soybeans (L’Hocine et al. 2006).

**Supercritical CO$_2$ (SC-CO$_2$) extraction**

In an attempt to eliminate organic solvent residue in extracted oil or meals used for human consumption, liquid or SC-CO$_2$ extraction has been investigated. These processes have the advantage in that CO$_2$ is easily removed from the meal, it is nontoxic, inexpensive, and non-polluting, and can be used for small-scale (~50 mt/d) or identity-preserved production unlike large (>3000 mt/d) solvent-extraction plants (Yu et al. 2007, Li et al. 2006, Stahl et al. 1980).

SC-CO$_2$ extraction can be used to extract isoflavones from soybean meals but results in lower total isoflavone yield when compared to solvent extraction, it is more applicable to the extraction of acetylglucoside and aglycone (Kao et al. 2008). Yu et al. (2007) were able to produce isoflavone-rich SPI from SC-CO$_2$ defatted soy meal. A 10% SC-CO$_2$/ethanol mixture was able to completely extract the phospholipids present in a defatted soybean meal (Montanari et al. 1997).

One disadvantage of using SC-CO$_2$ extraction is that soybean oil is not as soluble in SC-CO$_2$ compared to hexane (Li et al. 2006). The solubility of soy lecithin in SC-CO$_2$ increases as pressure increases when temperature is held constant. Oil solubility decreases with increasing temperature at constant pressure. Capital costs are quite high due to the need for high-pressure vessels and no means yet exists to get large volumes of solids into and out of the high-pressure vessels continuously. Therefore, only batch systems are available. SC-CO$_2$ has been commercially used for extracting high-value products such as in decaffeinated coffee and flavor concentrates.
Gas-supported screw-pressing (GSSP)

GSSP is a recently developed process by Crown Iron Works (St. Paul, MN, USA) and SafeSoy Technologies (Ellsworth, IA, USA). CO₂ is injected into a screw press as a displacement fluid to increase oil removal, thereby achieving low residual oil contents in meal (3-6% db). The CO₂ flashes when exiting the press to atmospheric pressure cooling the meal to achieve low protein denaturation and high PDIs (>70). GSSP meal was used to produce SPI in high yields and having unique functional properties (Deak et al. 2008).

Heat-treated SPIs

Inactivation of lipoxygenase and trypsin inhibitors (TI) using heat treatments denature and insolubilize proteins results in poor functionality (Kinsella 1979). Heat treatment causes dissociation, denaturation and aggregation of soy protein (Sorgentini et al. 1995).

Nakai and Lichan (1986) investigated heat treatments to improve SPI functional properties. Wang and Johnson (2001a) reported that hydrothermal cooking, a high-shear steam-infusion treatment, improved the functional properties of soy protein by disrupting large protein aggregates. Heat treatment in the presence of alkali improved some functional properties SPI, such as solubility and emulsification activity (Wu et al. 1999b). High-pressure treatment applied to SPI at an appropriate protein concentration can also be used to modify functional properties (Wang et al. 2008). SPIs treated with acid experienced structural changes and did not result in much improvement in functional properties. Exposure to mild acid for a specific time results in some denaturation of glycinin. Denaturation and dissociation results in reduced solubility, increased foaming capacity and stability (Wagner et al. 1996).
**Preservation**

No changes were observed in the subunit composition of soybeans stored either under mild, cold or ambient conditions and as a result no significant differences in functional properties of SPI were detected when compared to SPI from freezer-stored soybeans (Liu et al. 2008). Boatright and Hettiarachchy (1995) reported higher solubility of spray-dried SPIs compared to freeze-dried SPIs. Deak and Johnson (2007) studied the effects of spray-drying, freeze-drying and freezing-thawing on the functionality of SPIs. The preservation method significantly affected SPI functionality.

**Sensory properties**

Despite health benefits associated with consuming soy protein, soy protein ingredients are still not widely accepted due to poor sensory characteristics, caused mainly by off-flavors (MacLeod and Ames 1988). A number of studies have reported on hexanal (Fujimaki et al. 1965, Arai et al. 1970) in relation to the beany, grassy flavor it imparts to soybeans and soy products (Wilkens and Lin 1970, Solina et al. 2005). O'Keefe et al. (1991) reported on the number of sites in soy glycinin and β-conglycinin that bind to hexanal, which changes according to buffer conditions. They suggested that the binding of hexanal brings about structural changes to protein. Hexanal does not contribute to the beany aroma individually but does so in combination with other chemical compounds (Bott and Chambers 2006).

Other compounds responsible for the beany odor of soy include 1-hexanol, trans-2-nononal, 1-octen-3-ol, trans,trans-2-4-decadienal, trans,trans-2-4-nonadienal, acetophenone 2-pentyl pyridine and dimethyl trisulfide (Boatright and Lei 1999). Zhou and Cadwallader (2004) investigated the binding of hexane, 1-hexanol and hexanal to SPI under controlled relative humidity.
An earlier study (Mattick and Hand 1969) identified ethyl ketone to be responsible for the green bean odor and flavor of soy. Boatright and Lei (2000) determined the components responsible for odor of SPIs in solutions using static or dynamic headspace analyses with GC/MS techniques. This is the first study to report methanethiol to be one of the responsible odorants. 2-Pentyl pyridine (2-pp) is responsible for the strong grassy aroma detected by using GC and causes throat-catching taste (Boatright and Crum 1997). Anderson and Warner (1976) reported that acid-sensitive soy proteins had greater affinity for grassy-beany flavor. Kalbrener et al. (1974) observed that lipoxygenase hydroperoxides and their decomposition products contributed to grassy-beany flavor. Combining hexenal, methanethiol, 2-pentyl furan and dimethly trisulfide (DMTS) reproduced the odorants detected in the headspace atop an aqueous SPI slurry (Ang and Boatright 2003). Zhou and Boatright (1999) studied the effect of pH during SPI production on flavor due to 2-pp. They reported increased 2-pp levels at pH 7, which is lower at pH 4.5 or 9.

Other factors that contribute to the unfavorable flavor or soy products include bitterness and astringency. Arai et al. (1966) identified phenolic acids from defatted soy flour taste sour, bitter and astringent. Activated carbon and ion exchange removed the phenolic compounds from soy protein extracts, which improved flavor but did not improve bitterness or astringency (How and Morr 1982). Still other studies reported that soy isoflavones and soy saponins are responsible for astringent and/or bitter taste in soy products (Tsukamoto et al. 1995). Malonyl-β-glucoside isoflavones and DDMP-conjugated saponins cause bitterness and off-flavor (Aldin et al. 2006). Less processed or heat-treated soy products are more astringent. Other studies have suggested the bitter, rancid and beany off-flavors are caused by the oxidization of unsaturated fatty acids (Sessa and Rackis 1977). Sessa et al. (1976) isolated three phosphatidylcholines from residual lipid in hexane-defatted soy flakes, which contributes to the bitter taste of soy.
A number of attempts have been made to eliminate the objectionable beany flavor of soy products. Use of heat treatment has achieved little success. Breeding has also been used to eliminate lipoxygenase (Kitamura 1993), which reduced the beany flavor found in the soy products (Kobayashi et al. 1995). Extracting soy flakes with aqueous alcohol removed the objectionable flavors in soy (Baker et al. 1979). SPIs produced from defatted soy flakes and washed with aqueous alcohol had improved flavor profiles than SPIs produced from unwashed soy flakes (Hua et al. 2003). SPI produced from ethanol azeotrope-extracted flakes, toasted or untoasted, had less grassy or beany attributes but were still bitter in taste (Honig et al. 1976). A 66% reduction in beany flavor was reported in SPI produced from hexane/acetic-acid-treated soybean meal (Swamylingappa and Srinivas 1994). Maheshwari et al. (1995) reported that liquid carbon dioxide is the least effective and SC-CO₂ is most effective in removing volatile off-flavors from SPI. The azeotropic mixture of hexane and absolute ethanol produces flakes with little objectionable flavors by removing most residual lipids, which oxidize and lead to objectionable flavors (Sessa et al. 1969).

A descriptive sensory panel reported 19 different chemicals with beany aromas and flavors similar to that of soy. Beany flavor was described as musty/earthy, musty/dusty, sour aromatics, green/pod pea, nutty or even brown. Three alcohols, two ketones, one aldehyde and one pyrazine had beany characteristics at low concentrations (Vara-Ubol et al. 2004). Another descriptive sensory panel used the following descriptors to described commercial SPI samples in water (10% w/v) as cereal, malty, flour paste, roasted, sweet aromatic, cardboard and brothy flavor (Russell et al. 2006).

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CHAPTER 2. 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 CO₂, 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.

Keywords  CO₂ · Extraction · Protein functionality · Soybeans · Soy protein · Soy protein isolate.

**Introduction**

Soy protein isolate (SPI) is generally produced from solvent-extracted 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 dispersibility 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 CO₂ (SC-CO₂) a continuous process, SC-CO₂ has long been promoted as a means of extracting oil from soybeans to produce DSF. SC-CO₂ leaves very little residual CO₂ in the oil or meal and CO₂ is nonflammable, nontoxic [4] and economical [6]. SC-CO₂ 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-CO₂ 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 CO₂ 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 CO2 as an extraction aid in the new GSSP process may provide similar advantages as is achieved with SC-CO2.

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 the present 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 Crown Iron Works hot-dehulling system, flaked and screw pressed using Crown Iron Works Hyplex® screw-pressing process, which uses CO₂ 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 Figure 1, SPIs were prepared in the laboratory according to the methods of Deak and Johnson [8].

**Figure 1.** Soy protein isolation procedure

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). These values were converted to Kjeldal nitrogen concentrations using the conversion formula of Jung et al. [10]. The 6.25 x 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)] x 100. All measurements were replicated at least four times and 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 percentage points lower than for WFs (82) but were still reasonably high (58 to 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.

### Table 1. Compositions of partially defatted soybean flours used to produce SPIs

<table>
<thead>
<tr>
<th>Compositional Properties</th>
<th>Commodity</th>
<th>Organic</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WF (downdraft desolventized)</td>
<td>GSSP</td>
</tr>
<tr>
<td>Protein (% db)</td>
<td>54.8</td>
<td>53.2</td>
</tr>
<tr>
<td>Free Fat (% db)</td>
<td>1.6</td>
<td>6.5</td>
</tr>
<tr>
<td>Total Fat (% db)</td>
<td>2.5</td>
<td>6.9</td>
</tr>
<tr>
<td>PDI</td>
<td>81.7</td>
<td>68.4</td>
</tr>
</tbody>
</table>

*WF denotes white flakes and GSSP, gas-supported screw pressing.*

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.

Table 2. Yields and protein and fat contents of SPIa

<table>
<thead>
<tr>
<th>Defatted Soy Flour Used to Prepare SPI</th>
<th>Solids Yield (%)</th>
<th>Protein Yield (%)</th>
<th>Protein Content (%)</th>
<th>Free Fat Content (%)</th>
<th>Total Fat Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDWF</td>
<td>30.4c</td>
<td>51.6c</td>
<td>92.9a</td>
<td>0.12b</td>
<td>2.40c</td>
</tr>
<tr>
<td>CGSSP</td>
<td>34.6b</td>
<td>59.7b</td>
<td>91.8b</td>
<td>0.40a</td>
<td>4.92a</td>
</tr>
<tr>
<td>OADWF</td>
<td>38.0a</td>
<td>61.1b</td>
<td>93.2a</td>
<td>Nd</td>
<td>1.82d</td>
</tr>
<tr>
<td>OGSSP</td>
<td>36.8ab</td>
<td>63.1a</td>
<td>94.6a</td>
<td>0.05b</td>
<td>3.70b</td>
</tr>
</tbody>
</table>

aN = 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.

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.

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 water wash the SPIs as is sometimes done in commercial practice, which would reduce 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 \(\beta\)-conglycinin composition of the WF and GSSP SPIs.

**Table 3. Individual protein compositions of SPI (% of total protein)**

<table>
<thead>
<tr>
<th>Defatted Soy Flour Used to Prepare SPI</th>
<th>Lipoxygenase</th>
<th>(\beta)-Conglycinin</th>
<th>Glycinin</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDWF</td>
<td>6(^a)</td>
<td>45(^b)</td>
<td>42(^{ab})</td>
<td>8(^b)</td>
</tr>
<tr>
<td>CGSSP</td>
<td>Nd</td>
<td>47(^{ab})</td>
<td>43(^a)</td>
<td>10(^a)</td>
</tr>
<tr>
<td>OADWF</td>
<td>4(^a)</td>
<td>49(^a)</td>
<td>39(^b)</td>
<td>8(^b)</td>
</tr>
<tr>
<td>OGSSP</td>
<td>Nd</td>
<td>48(^a)</td>
<td>41(^{ab})</td>
<td>11(^a)</td>
</tr>
</tbody>
</table>

\(^{a}n = 3\). 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.

**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).

**Table 4. Thermal behaviors of SPIs**

<table>
<thead>
<tr>
<th>Defatted Soy Flour Used to Prepare SPI</th>
<th>(\beta)-Conglycinin</th>
<th>Glycinin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>On set Td ((^\circ)C)</td>
<td>Off set Td ((^\circ)C)</td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-----------------------</td>
<td>-----------------------</td>
</tr>
<tr>
<td>CDDWF</td>
<td>66.4(^a)</td>
<td>82.4(^a)</td>
</tr>
<tr>
<td>CGSSP</td>
<td>69.1(^a)</td>
<td>81.1(^a)</td>
</tr>
<tr>
<td>OADWF</td>
<td>70.2(^a)</td>
<td>83.4(^a)</td>
</tr>
<tr>
<td>OGSSP</td>
<td>68.8(^a)</td>
<td>81.6(^a)</td>
</tr>
</tbody>
</table>

\(^{a}n = 3\). 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.
pressed commodity soybeans; OADWF, hexane-extracted, air-desolventized white flakes from organic soybeans; and OGSSSP, gas-supported screw-pressed organic soybeans.

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 ethalpies for SPIs prepared from GSSP meals were higher than for the glycinin in SPIs prepared from WFs. The enthalpies for $\beta$-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 to 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].

### Table 5. Solubilities, water-holding capacities and dynamic viscosities of SPIs

<table>
<thead>
<tr>
<th>Defatted Soy Flour Used to Prepare SPI</th>
<th>Solubility (%)</th>
<th>Water-holding Capacity (g water/g sample)</th>
<th>Flow Consistency Index (K, mPa*s)</th>
<th>Flow Behavior Index (n, dimensionless)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDWF</td>
<td>89.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.190&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.669&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CGSSP</td>
<td>82.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.356&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.596&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>OADWF</td>
<td>91.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.4&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.256&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.638&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>OGSSP</td>
<td>88.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.616&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.511&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
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.

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 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 to 4.4 g water/g protein for SPIs prepared from GSSP meals were similar to the WHCs for EE-processed soy flours (3.7 to 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 shape of the proteins is 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 WF (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 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 6). 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.

<table>
<thead>
<tr>
<th>Defatted Soy Flour Used to Prepare SPI</th>
<th>Emulsification Capacity (g oil/g sample)</th>
<th>Emulsification Activity (A% at 500 nm)</th>
<th>Emulsification Stability Index (dimensionless)</th>
<th>Fat-binding Capacity (g fat/g sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDWF</td>
<td>510d</td>
<td>34.7c</td>
<td>142b</td>
<td>3.3b</td>
</tr>
<tr>
<td>CGSSP</td>
<td>678b</td>
<td>44.5a</td>
<td>279a</td>
<td>3.6a</td>
</tr>
<tr>
<td>OADWF</td>
<td>607c</td>
<td>38.1b</td>
<td>154b</td>
<td>3.0b</td>
</tr>
<tr>
<td>OGSSP</td>
<td>743a</td>
<td>47.0a</td>
<td>277a</td>
<td>3.7a</td>
</tr>
</tbody>
</table>

*a = 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.

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 EAs and ESIIs 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 ESIIs 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 denaturates in GSSP vs WFs as has been previously discussed.

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 vs commodity beans, but the organic soybeans produced SPIs with faster rates of foaming than did SPIs produced from commodity beans.

Table 7. Foaming properties of SPIs

<table>
<thead>
<tr>
<th>Defatted Soy Flour Used to Prepare SPI</th>
<th>Foaming Capacity (mL/mL)</th>
<th>Rate of Foaming (mL/min)</th>
<th>Foaming Stability (1/K=mL*min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDDWF</td>
<td>1.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CGSSP</td>
<td>0.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OADWF</td>
<td>1.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>OGSSP</td>
<td>0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*<sup>a</sup>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 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.

**Acknowledgments**

The authors gratefully acknowledge the support of the Grow Iowa Values Fund and the Iowa Agricultural and Home Economics Experiment Station.

**References**


CHAPTER 3. FUNCTIONAL PROPERTIES OF JET-COOKED AND HYDROGEN-PEROXIDE-TREATED SOY PROTEIN ISOLATES

A paper to be submitted to the Journal of American Oil Chemists Society

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Center for Crops Utilization Research
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Abstract

Gas-supported screw pressing (GSSP) is a new crushing technology where traditional screw-pressing is combined with injection of high-pressure CO₂, producing a hexane-free soybean meal suitable for making organic SPI. Jet-cooking (JC) and hydrogen peroxide (H₂O₂) are preservation treatments that can be used to control the microbiological safety of soy protein isolate (SPI). The effects of these preservation and oil extraction methods on the functional properties of SPI were evaluated. H₂O₂-treated SPI prepared from hexane-extracted white flakes (WFs) was the most soluble, while jet-cooked SPI prepared from GSSP meal was the least soluble. Jet-cooking nearly completely denatured β-conglycinin, while leaving some residual glycinin. H₂O₂ did not denature glycinin, but partially denatured β-conglycinin. Jet-cooked SPIs had greater water-holding capacities than other treatments and gave higher viscosities. Untreated SPIs from WFs had the best emulsifying capacities. Untreated SPIs had significantly better fat-binding capacities. SPIs prepared from WFs had better foaming rates and foam stabilities. SPIs prepared from GSSP did not differ in functionality from SPIs prepared from WFs, except for having lower solubility and foaming abilities. Using H₂O₂ as a preservative improved solubility, reduced glycinin and β-conglycinin denaturation, and improved emulsification and foaming properties of SPI.
**Keywords**  CO₂ · Extraction · Jet-cooking · Hydrogen peroxide · Protein functionality · Screw pressing · Soybeans · Soy protein · Soy protein isolate.

**Introduction**

Environmentally friendly processes are being developed to extract oil from soybeans. Supercritical fluid extraction using CO₂ has been shown to be beneficial in producing defatted soy meal with high PDI (protein dispersibility index) indicating little protein denaturation [1]. Soy protein products with high solubility and good flavor were produced by using supercritical CO₂ extracted meal [2], but this is an expensive process and challenges remain about how to continuously transport solid material into and out of a pressurized extraction vessel. As an alternative to the traditional solvent-extraction process, a new crushing technology known as gas-supported screw pressing (GSSP) has been developed by Crown Iron Works (Minneapolis, MN, USA) under the trade name of Hiplex®. This process removes oil from the soybeans by injecting CO₂ into a screw press. The process is not believed to generate sufficient pressures to achieve the supercritical state, rather the CO₂ is believed to act as oil displacement fluid achieving lower residual oil contents in the meal than normal screw pressing and acting as a cooling agent minimizing protein denaturation. In addition to being nontoxic and inexpensive, using CO₂ eliminates any organinc solvent residue in soybean meal, making this a “green” or “organic” process.

Food safety a major concern in today’s global market makes food preservation an important aspect in the processing and production of soy protein isolate (SPI). Consumers demand high quality food that is microbiologically safe and minimally processed. SPI is gaining importance as an economical, functional, high-quality protein ingredient. SPI is often exposed to high temperatures during manufacture, affecting functional properties important to manufacturing food. Desolventization during oil extraction prior to manufacturing SPI also exposes the protein to high temperatures. The most common means of preserving SPI and preventing microbial growth is jet-cooking just prior to spray-drying. In jet-cooking, steam is
injected under pressure directly into the SPI slurry to achieve high temperature (120-150°C) for short time (10-60 sec) eliminating any potentially harmful microorganisms that may be present. These exposures to heat bring about changes in the functionality of the proteins, due to denaturation [3]. Hence, it is desirable to have alternative oil extraction and SPI preservation treatments that have less detrimental effects on protein functionality.

Hydrogen peroxide is a strong oxidative compound that is used to sanitize food processing equipment and food packaging. Food-grade H\textsubscript{2}O\textsubscript{2} is generally recognized as safe (GRAS) in food at low concentrations and is used as an antimicrobial agent in milk for cheese-making, whey, corn starch and dried eggs [4] and is also an effective sanitizer for use on fruits and vegetables [5]. We recently found 0.06-0.1% H\textsubscript{2}O\textsubscript{2} to be as effective as jet-cooking in reducing microbial loads in SPI while causing less protein denaturation and improving functional properties [6].

Many applications for SPI in foods are driven by functional properties. Based on our previous laboratory study [7], we hypothesized that SPI prepared from GSSP soybean meal and preserved with H\textsubscript{2}O\textsubscript{2} would have improved functional properties to SPI prepared from WFs and preserved by jet-cooking. The objectives of our present study were to evaluate and compare functional properties of SPI produced from GSSP soybean meal to that of SPI produced from WFs at pilot-plant scale and to compare H\textsubscript{2}O\textsubscript{2} to jet-cooking as a preservative treatment for SPIs.

**Experimental Procedures**

**Materials**

Soy flours were produced using two different oil-extraction methods. The hexane-extracted flash-desolventized WFs were processed and supplied by Cargill Inc (Minneapolis, MN, USA). The GSSP meal was also processed and supplied by Crown Iron Works (Minneapolis, MN, USA). The beans were dehulled using Crown Iron Works hot-dehulling
system, flaked and screw pressed using Crown Iron Works Hiplex® gas-supported screw-press process. Upon receipt, all partially defatted meals were ground into 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 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**

Solvent-extracted WFs and GSSP-extracted soybean meals were used to prepare isoelectric-precipitated SPI. SPIs were prepared in the pilot plants of the Center for Crops Utilization Research (Iowa State University) according to the methods of Deak and Johnson [8]. Neutralized SPI slurries (~10% solids) were divided into three portions. One portion was left untreated (raw), one was jet-cooked using a Pick jet-cooker (model SC2-3 pilot; West Bend, WI, USA) with a holding time of 17 sec at 105°C and then homogenized by using a Stephan Microcut Mill (model MC-10; Stephan Machinery Corp., Columbus, OH, USA) with a 0.2-mm cutting-ring; another portion was treated with 0.1% H₂O₂ (30%, ACS certified, Fisher Chemicals, Pittsburgh, PA, USA). All three SPI slurries were spray-dried with an APV Crepaco spray-dryer (Model Lab; Attleboro Falls, MA, USA). Air inlet and outlet temperatures were 165°C and 90-95°C, respectively. SPIs were prepared in duplicate using 50 Kg of DSF prepared from WFs and GSSP soybean meal.

**Proximate Analyses and Mass Balance**

The nitrogen contents of all samples were measured by using the combustion or Dumas method [9] with a Rapid NIII Analyzer (Elmentar Americas, Inc., Mt Laurel, NJ, USA). These values were converted to Kjeldhal nitrogen concentrations using the conversion formula of Jung et al. [10]. The conversion factor 6.25 x Kjeldhal N was used to convert percentage of
nitrogen to protein content. PDI was determined by N-PAL (St. Louis, MO, USA). Mass balances for protein and solids (dry matter) were determined for SPI production from both WFs and GSSP meal. Moisture content was determined by oven drying for 3 h at 130°C [9]. 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 proteins 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)] x 100. All measurements were replicated at least four times and means reported.

**Functionality**

Thermal behavior, solubility, and 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 the desired value 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**

Protein content of the starting WFs was higher than the GSSP meal by four percentage points (Table 1). The total fat contents (3.7-7.3% db) of both starting materials were significantly higher than their free fat contents (0.9-6.5% db).

<table>
<thead>
<tr>
<th>Compositional Properties</th>
<th>WFs</th>
<th>GSSP Meal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (% db)</td>
<td>56.3</td>
<td>51.78</td>
</tr>
<tr>
<td>Free Fat (% db)</td>
<td>0.9</td>
<td>6.5</td>
</tr>
<tr>
<td>Total Fat (% db)</td>
<td>3.7</td>
<td>7.3</td>
</tr>
<tr>
<td>PDI</td>
<td>81.7</td>
<td>68.4</td>
</tr>
</tbody>
</table>

*WFs denotes white flakes and GSSP, gas-supported screw pressing.*

The GSSP meal contained significantly more fat (6.5-7.3% db) than the WFs (0.9-3.7% db). The WF had higher PDI than the GSSP meal, but the GSSP meal had a reasonably high PDI, much higher than for normal screw-pressed meal (10.6) [17]. The compositions of the starting materials were similar to those used in our previous study [7]; but, unlike our laboratory study, the yields of solids and protein as SPI from GSSP meals were lower than those of SPIs made from WFs (Table 2). The GSSP meals had lower PDIs than WFs. The WF
SPI contained more protein than GSSP SPI, which was due to the higher fat content of the GSSP SPI. Unlike the >90% protein content SPIs we obtained in laboratory SPI production [7], pilot plant production resulted in SPIs with lower protein contents (80.2-84.4%).

**Table 2.** Yields and protein and fat contents of SPIs prepared by different oil-extraction methods

<table>
<thead>
<tr>
<th>Defatted Soy Material Used to Prepare SPI</th>
<th>Solids Yield (%)</th>
<th>Protein Yield (%)</th>
<th>Protein Content (%)</th>
<th>Free Fat Content (%)</th>
<th>Total Fat Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFs</td>
<td>40.05</td>
<td>60.04</td>
<td>84.4</td>
<td>0.12</td>
<td>2.6</td>
</tr>
<tr>
<td>GSSP Meal</td>
<td>37.15</td>
<td>57.54</td>
<td>80.2</td>
<td>0.48</td>
<td>6.34</td>
</tr>
</tbody>
</table>

*WFs denotes white flakes and GSSP, gas-supported screw pressing.

**Compositions of Individual Proteins**

Lipoxygenase was not detected in the SPI prepared from GSSP meal but was present in the SPI prepared from WFs despite the GSSP meal containing as much lipoxygenase as the WFs (Table 3). This was consistent with our previous lab findings [7]. Unlike industry practice, we did not wash the SPIs with water; hence, water washing was not responsible for these differences. Both SPI prepared from WFs and SPI prepared from GSSP meal contained more glycinin than β-conglycinin.

**Table 3.** Individual protein compositions of SPI (% of total protein) prepared by different oil-extraction and preservation methods

<table>
<thead>
<tr>
<th>Material</th>
<th>Lipoxygenase</th>
<th>β-Conglycinin</th>
<th>Glycinin</th>
<th>Other Proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFs</td>
<td>7a</td>
<td>34a</td>
<td>52c</td>
<td>8b</td>
</tr>
<tr>
<td>WF SPI</td>
<td>0.2b</td>
<td>23b</td>
<td>69a</td>
<td>8b</td>
</tr>
<tr>
<td>GSSP Meal</td>
<td>6a</td>
<td>26b</td>
<td>55b</td>
<td>13a</td>
</tr>
<tr>
<td>GSSP SPI</td>
<td>Nd</td>
<td>31a</td>
<td>68a</td>
<td>2c</td>
</tr>
</tbody>
</table>

*n = 3. Means within a column for a specific sample followed by different superscripts are significantly different at *p* <0.05. WFs denotes white flakes and GSSP, gas-supported screw pressing and nd, non-detectable.
Solubility Profile

Low solubility can limit potential end-uses for soy protein, making protein solubility an important functional property. Heat applied to soy protein during dehulling, conditioning, defatting, desolventization, protein extraction, cooking or drying can reduce protein solubility, however, solubility, cannot be used as a definitive indicator of extent of protein denaturation caused by heat [3].

Figure 1. Solubilities and water-holding capacities of SPIs prepared by different oil-extraction and preservation methods

\(^a\)\(n=3\). Points within a pH with different superscripts are significantly different at \(p<0.05\). WFs denotes white flakes and GSSP, gas-supported screw pressing.
SPIs prepared from WFs had slightly greater solubilities than SPIs prepared from GSSP meal (Figure 1, A and B), indicating that oil-extraction method affects protein solubility. H2O2 treatment produced SPIs prepared from WFs with high solubility compared to jet-cooking. Jet-cooked SPIs had the lowest solubilities using either WFs or GSSP meal. Jet-cooking reduced the solubilities of SPI at all pHs except pH 9. The raw SPI prepared from GSSP meal had the greatest solubility, except at pH 5 where H2O2-treated SPI had significantly higher solubility.

**Thermal Behavior of SPI**

Differential scanning calorimetry is a more reliable indicator of protein denaturation than solubility. There were no differences in total β-conglycinin denaturation enthalpies (Table 4) indicating that the β-conglycinin present in the GSSP meal was equally denatured as in WFS. The glycinin enthalpies were higher than the β-conglycinin enthalpies for all treatments, which was expected because SDS-PAGE indicated higher amounts of glycinin were present. There were no significant differences in the β-conglycinin enthalpies among treatments. Jet-cooked SPIs had the lowest glycinin enthalpy, followed by H2O2-treated SPIs and then the raw SPIs with the highest glycinin enthalpy. The untreated SPIs contained significantly higher glycinin enthalpies for both SPI produced from WFs and SPI produced from GSSP meal. This indicated that jet-cooking significantly denatured most of the glycinin in SPI. About one-half the enthalpy for glycinin and about three-fourths the enthalpy for β-conglycinin remained in the H2O2-treated SPIs. There were no significant differences in peak onset, off set and peak temperatures among SPIs produced by the different oil-extraction processes or different preservation treatments.
<table>
<thead>
<tr>
<th>Material Used to Prepare SPI</th>
<th>Preservation Treatment</th>
<th>β-Conglycinin</th>
<th>Glycinin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Onset Td (°C)</td>
<td>Offset Td (°C)</td>
</tr>
<tr>
<td>Raw</td>
<td></td>
<td>69.6a</td>
<td>81.1b</td>
</tr>
<tr>
<td>Jet-cooking</td>
<td></td>
<td>69.3b</td>
<td>79.9b</td>
</tr>
<tr>
<td>H₂O₂</td>
<td></td>
<td>69.9a</td>
<td>81.3b</td>
</tr>
<tr>
<td>Raw</td>
<td></td>
<td>70.4a</td>
<td>83.4a</td>
</tr>
<tr>
<td>Jet-cooking</td>
<td></td>
<td>69.9a</td>
<td>79.8b</td>
</tr>
<tr>
<td>H₂O₂</td>
<td></td>
<td>70.4a</td>
<td>81.9ab</td>
</tr>
</tbody>
</table>

\(^a_n = 3\). Means within a column for a specific sample followed by different superscripts are significantly different at \(p < 0.05\). WFs denotes white flakes and GSSP, gas-supported screw pressing.

**Water-holding Capacity (WHC)**

WHC is the ability of SPI to hold water against gravity [3], which is important to reducing cooking losses in food. SPIs prepared from GSSP meal had similar WHC to SPIs prepared from WFs except at a few pHs (Figure 1, C and D). Jet-cooking, however, significantly increased the WHC of both SPI prepared from WFs and SPI prepared from GSSP meal. H₂O₂ treatment did not affect the WHC of SPI prepared from WFs compared to the raw SPI prepared from WFs, but did improve the WHC of the SPI prepared from GSSP meal compared to the raw SPI prepared from GSSP meal although not significantly.

**Dynamic Viscosity**

Viscosity is important to the texture and mouthfeel of foods, especially beverages, and is affected by denaturation and unfolding of the protein due to heat and pH [3]. 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.

**Table 5.** Dynamic viscosities and fat-binding properties of SPIs prepared by different oil-extraction and preservation methods

<table>
<thead>
<tr>
<th>Material Used to Prepare SPI</th>
<th>Preservation Treatment</th>
<th>Flow Consistency Index ((k = \text{mPa*s}))</th>
<th>Flow Behavior Index ((n, \text{dimensionless}))</th>
<th>Fat-binding Capacity ((\text{g fat/g sample}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFs</td>
<td>Raw</td>
<td>0.10(^b)</td>
<td>0.76(^a)</td>
<td>5.4(^a)</td>
</tr>
<tr>
<td></td>
<td>Jet-cooking</td>
<td>2.80(^a)</td>
<td>0.46(^b)</td>
<td>2.2(^b)</td>
</tr>
<tr>
<td></td>
<td>(\text{H}_2\text{O}_2)</td>
<td>0.12(^b)</td>
<td>0.72(^a)</td>
<td>2.4(^b)</td>
</tr>
<tr>
<td>GSSP Meal</td>
<td>Raw</td>
<td>0.77(^b)</td>
<td>0.58(^ab)</td>
<td>5.5(^a)</td>
</tr>
<tr>
<td></td>
<td>Jet-cooking</td>
<td>3.10(^a)</td>
<td>0.49(^b)</td>
<td>2.7(^b)</td>
</tr>
<tr>
<td></td>
<td>(\text{H}_2\text{O}_2)</td>
<td>0.87(^b)</td>
<td>0.59(^ab)</td>
<td>2.2(^b)</td>
</tr>
</tbody>
</table>

\(^a\)\(n = 3\). Means within a column for a specific sample followed by different superscripts are significantly different at \(p < 0.05\). WFs denotes white flakes and GSSP, gas-supported screw pressing.

There were no significant differences in the flow consistency and flow behavior indexes between SPIs prepared from WFs and GSSP meal (Table 5); oil-extraction process had no effect on dynamic viscosity. Jet-cooking significantly increased the flow consistency index of both SPIs prepared from both WFs and GSSP meal, indicating that jet-cooking significantly increases viscosity. In the thermal behavior studies we observed, jet-cooked SPIs had less native glycinin and \(\beta\)-conglycinin. Pertrucelli [18] reported viscosity decreased as solubility increased, which was not consistent with our results. \(\text{H}_2\text{O}_2\) treatment significantly reduced the flow consistency index for both SPI prepared from WFs and GSSP meal compared to jet-cooking. The untreated and \(\text{H}_2\text{O}_2\)-treated SPIs prepared from WFs had significantly higher flow behavior indexes values than those of jet-cooked SPI prepared from WFs, indicating they were less viscous.
Fat-binding Capacity (FBC)

There were no differences in FBC due to oil-extraction process (Table 5). Unlike our laboratory studies [7] that showed SPI prepared from GSSP meal had significantly greater FBC compared to SPI prepared from WFs, there were no differences in FBC among SPIs prepared from GSSP meal and WFs. The untreated SPIs had significantly greater FBC compared to the treated SPIs. Jet-cooking and H₂O₂ treatments had similar effects on both SPIs prepared from WFs and GSSP meal, significantly reducing FBC.

Emulsification Properties

The flexibility of the protein determines how well it can reduce the surface tension in an oil-water system. Protein flexibility is determined by the protein’s ability to unfold and expose hydrophobic regions to lipid [3]. L’Hocine’s [19] findings showed that defatting process affects emulsification capacity (EC), but this is not consistent with our present findings. No significant differences in EC were detected between the SPIs produced from WFs and GSSP meal among any treatment (Figure 2, A and B).

Contrary to expectations, the higher fat content of GSSP meal did not affect EC of SPI. Both untreated SPIs prepared from WFs and GSSP meals showed greater EC, compared to jet-cooked and H₂O₂-treated SPIs. Jet-cooking reduced the ECs of both SPIs prepared from WFs and GSSP meal compared to the H₂O₂-treated SPIs, except at pH >6. H₂O₂ treatment gave better ECs for both SPIs prepared from WFs and GSSP meal.

The emulsifying activity (EA) of H₂O₂-treated SPI prepared from WFs was better than for other treatments, except at pH 2 (Figure 2, C and D). Jet-cooking and H₂O₂ treatment on GSSP SPI prepared from GSSP meal improved EA, except at the isoelectric point (pH 4). The emulsification stability index (ESI) of SPI prepared from WFs was not significantly different from any treatments at any pH (Figure 2, E and F).
**Figure 2.** Emulsification properties of SPIs prepared by different oil-extraction and preservation methods

**A**

**B**

**C**

**D**

**E**

**F**

*a* n=3. Points within a pH with a different superscripts are significantly different at *p*<0.05. WF denotes white flakes and GSSP, gas-supported screw pressing.
Jet-cooking SPI prepared from GSSP meal significantly increased ESI at pH 6, 7 and 9. H$_2$O$_2$-treated SPI prepared from GSSP meal showed some increase in ESI but was not significantly different from the untreated SPI prepared from GSSP meal.

**Foaming Properties**

To form stable foams, the proteins hydrophobic regions need to be exposed and form a film around gas droplets. This requires the protein to unfold and associate with polypeptides to form a continuous film. The higher fat content of the GSSP SPI did not affect its foaming capacity as found in our laboratory study [7]. No significant differences were found in foaming capacity (FC) between WF SPI and GSSP SPI (Figure 3, A and B). H$_2$O$_2$-treated SPIs had slightly greater increase in FC compared to the jet-cooked and untreated SPIs at pH 5 and 9 for the WFs and pH 6 and 9 for GSSP meal. At pH 9 the FCs of the GSSP SPIs decreased. GSSP SPI had lower foam stability indexes (FSIs) than WF SPI (Figure 3, C and D), which we attributed to the high fat content.

The FSIs of H$_2$O$_2$-treated SPIs for both WFs and GSSP were similar to, if not better than, the FSIs of jet-cooked SPIs. WF SPIs had a higher rate of foaming than the GSSP SPIs (Figure 3, E and F). H$_2$O$_2$-treated SPIs had higher rates of foaming than the untreated or jet-cooked SPIs at most pHs.
Figure 3. Foaming properties of SPIs prepared by different oil-extraction and preservation methods.

A

B

C

D

E

F

\(^n=3.\) Points within a pH with different superscripts are significantly different at \(p<0.05.\) WFs denotes white flakes and GSSP, gas-supported screw pressing.
Conclusions

GSSP is a solvent-free process that can be used to produce SPI on a large scale with good functional properties. SPI produced from GSSP meal has solubility, WHC, viscosity, FBC, emulsification properties similar to SPI from hexane-extracted WFs. SPI prepared from GSSP meal forms less stable foams due to its high fat content. These results differed with findings of our previous study. We attributed this to the fact that the SPIs in the present study were produced on a larger scale and were spray-dried as industry would do. Our previous study was done in a laboratory and the SPIs were freeze dried. Using \( \text{H}_2\text{O}_2 \) as a preservative may prove to be a better alternative than jet-cooking for producing a safe and functional SPI. Jet-cooking denatured more glycinin than treating with \( \text{H}_2\text{O}_2 \). The solubilities of the \( \text{H}_2\text{O}_2 \)-treated SPIs were equivalent to or better than jet-cooked SPIs. \( \text{H}_2\text{O}_2 \)-treated SPIs had similar WHC, viscosity and FBC to raw SPIs. \( \text{H}_2\text{O}_2 \) treatment gave SPIs better emulsification and foaming properties than jet-cooking. SPIs produced from GSSP meal can become a valuable “organic” ingredient that can be used instead of SPIs prepared from traditional solvent-extracted WFs.

Acknowledgments

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References


   http://www.foodsciencecentral.com/library.html#fis/12433


CHAPTER 4. SENSORY PROPERTIES OF SOY PROTEIN ISOLATE AND GLYCININ-RICH AND β-CONGLYCININ-RICH FRACTIONS PREPARED FROM GAS-SUPPORTED SCREW-PRESSED SOYBEAN MEAL

A paper to be submitted to the *Journal of American Oil Chemists Society*

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Abstract

Gas-supported screw pressing (GSSP) is a new extraction process that uses high-pressure CO$_2$ in combination with screw pressing to produce solvent-free soybean meal. The effects of oil extraction method on the sensory properties of soy protein isolate (SPI) and glycinin-rich (gly) and β-conglycinin-rich (β-con) fractions prepared from GSSP meal and hexane-extracted white flakes (WFs) were evaluated. A descriptive sensory panel with 12 trained panelists developed terms to describe the aroma, flavor and mouthfeel of the soy protein solutions. The protein products prepared from GSSP soybean meal had similar sensory properties to those produced from WF, except for having greater mouthcoating. The gly-rich and β-con-rich fractions had significantly stronger fishy aromas, less floury aromas, less raw beany aroma and less floury flavor than the SPIs. SPI had significantly less astringent mouthfeel than the gly-rich and β-con-rich fractions. There were no sensory differences between the SPI and the gly-rich and β-con-rich fractions for cooked beany flavor and mouthcoating. The protein products prepared from WFs and GSSP soybean meal were
similar in L-value and –a value, but protein products prepared from the GSSP soybean meal had higher +b value. SPI had significantly lower L-value than the gly-rich and β-con-rich fractions.

**Keywords**  CO₂ · Extraction · Soybeans · Soy protein · Glycinin · β-conglycinin · Soy protein isolate · screw pressing · gas-supported screw pressing · Descriptive sensory analyses.

**Introduction**  
Sensory properties of soy protein ingredients are important in determining the use and acceptance of food products in which they are used. Soy proteins have health benefits but these ingredients are not widely accepted due to off-flavors and aromas. The soy protein industry is continually striving to gain a better understanding of soy protein and the effects processing has on its sensory properties. Unprocessed soybeans are generally bland or have mild flavor [1]. Off-flavors often referred to as “beany” and “green beany” [2] are attributed to interactions between soy protein and flavor compounds during processing [3]. Some sources of the off-flavors in soy protein originate with lipid oxidation, lipoxygenase activity, polar lipids, bitter peptides and lipids [1]. Many processes have been implemented to alleviate these problems including the use of heat, enzymes, and extraction with organic solvents and supercritical CO₂ (SC-CO₂).

SC-CO₂ produces soy protein products in the laboratory with high solubility and good flavor [4]. In a recent study, Maheshwari and others [5] found SC-CO₂ treatment to be effective in removing volatile off-flavors from soy protein isolate (SPI). This process, however, is not only expensive due to high capital costs but is challenging to use on a large scale because of inability to make the process continuous.

Soy protein ingredients are generally produced from solvent-extracted soybean white flakes (WFs) or defatted soy flour (DSF). The solvent hexane is used to extract crude oil and
the defatted meal is desolventized by either flash- or downdraft-desolventizing to keep protein denaturation to a minimum [6]. WFs undergo minimal protein denaturation and have good protein dispersibility (PDI) or nitrogen solubility indexes (NSI). There are, however, concerns about the cost, flammability, availability, [7], greenhouse gas emissions, high capital costs and potential toxicity of hexane [8].

Crown Iron Works (Minneapolis, MN, USA) developed new soybean crushing technologies under the trade name of Hiplex®. This gas-supported screw pressing (GSSP) process extracts oil from the soybeans by injecting liquefied CO₂ into a screw press. CO₂ behaves as an oil-displacing fluid and minimizes protein denaturation by acting as a cooling agent when the CO₂ flashes as it exits the pressing chamber. This process does not leave any harmful solvent residue in the soybean meal. Our previous studies have reported the compositional and functional properties of proteins made from this new GSSP process [9,10]. GSSP meals are lower in protein content due to its higher fat content with similar PDIs to the WFs. Laboratory-produced GSSP protein ingredients had higher water-holding capacities and viscosities, and significantly better emulsifying and fat-binding properties compared to SPIs prepared from WFs [9]. When produced at pilot-plant scale, SPIs prepared from GSSP meal did not differ in functionality from SPIs prepared from WFs, except for having slightly lower solubility and foaming abilities [10].

Little is known about the effects of oil-extraction method on the sensory properties of soy protein products. While technologies to make SPI (>90% protein db) have been practiced for many years, only recently has a process been developed to economically recover fractionated protein products [11-13]. Therefore, it is important to investigate the sensory properties of SPI and the major soy storage proteins glycinin (gly) and β-conglycinin (β-con).

In the present work, soy protein ingredients were produced from soybean meals extracted using hexane extraction with downdraft desolventization and GSSP to determine processing effects on the aroma, flavor and mouthfeel of soy protein ingredients prepared
from WFs and GSSP meal. The objectives of the present study were to: (1) determine if the sensory properties of soy protein products produced from GSSP meal were different from those produced from WFs; and (2) to determine if the sensory properties of the SPIs were different from those of the glycinin-rich and β-conglycinin-rich protein fractions.

**Experimental Procedures**

**Materials**

DFSs were produced from the two different extraction methods. The hexane-extracted, flash-desolventized WFs were processed and supplied by Cargill Inc (Minneapolis, MN, USA). The GSSP meal was processed and supplied by Crown Iron Works (St. Paul, MN, USA). The beans were dehulled using Crown Iron Works hot-dehulling system, flaked and screw-pressed using Crown Iron Works Hiplex® gas-supported screw-press process in their pilot plant located in St. Paul, MN, USA. Upon receipt, all partially defatted meals were ground into defatted 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 preserve the native protein state. The DSFs were stored in sealed containers and kept at 4ºC until used. The compositions of the GSSP meals and WFs are shown in Table 1.

**SPI, Glycinin and β-Conglycinin Production**

WFs and GSSP-extracted soybean meals were used to prepare isoelectric SPI and gly-rich and β-con-rich fractions in the pilot plant of the Center for Crops Utilization Research (Iowa State University) according to the methods of Deak and Johnson [11,12] and Deak et al. [13,14]. Neutralized soy protein slurries (~10% solids) were jet-cooked by using a Pick jet-cooker (model SC2-3 pilot; West Bend, WI, USA) at 105ºC for 17 sec and then
homogenized by using a Stephan Microcut (Mill) homogenizer (model MC-10; Stephan Machinery Corp., Columbus, OH, USA) with a 0.2-mm cutting-ring. All protein slurries were spray-dried using an APV Crepaco spray-dryer (model Lab; Attleboro Falls, MA, USA). Air inlet and outlet temperatures were 165°C and 90-95°C, respectively. SPIs and glyc-rich and β-con-rich fractions were prepared in duplicate from DSFs of both WFs and GSSP soybean meal.

**Proximate Analyses**

Moisture content was determined by oven drying for 3 h at 130°C [15]. The nitrogen contents were measured by using the combustion or Dumas method [15] with a Macro Elemental Analyzer (Elmentar Americas, Inc., Mt Laurel, NJ, USA). The conversion factor 6.25 x Kjeldhal N was used to convert percentage nitrogen to protein content. These values were converted to Kjeldhal nitrogen concentrations using the conversion formula of Jung et al. [16].

Crude free fat contents were determined by using the Goldfisch extraction procedure [17]. Total fat (free plus bound lipid) was determined by using the Mojonnier acid hydrolysis method [15]. Each sample was analyzed in triplicate and means reported.

**Protein Compositions**

Urea-SDS-PAGE gel electrophoresis was used to quantify the individual protein components by using the methods of Wu [18]. Lipoxygenase and soybean storage protein bands were identified by using a pre-stained SDS-PAGE MW standard, broad range (Bio-Rad Laboratories, Hercules, CA, USA). 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 means reported.

Selection and Training of Panelists

Panelists (n=12) were selected from Iowa State University students volunteering to be on the panel. The selection process consisted of a pre-screening questionnaire [19] and a taste recognition test according to procedures described by ASTM [20]. The panelists were selected based on their willingness to consume soy products, lack of health issues that could affect their perception of flavors or odors, ability to articulate observations and follow instructions, and availability.

The participants were trained in two 60-min sessions (two days each week) for 5 wks with sample evaluations occurring in the 6th week. Time and days for sensory training and evaluation were decided based on the availability of the panelists. The training sessions were conducted in a roundtable setting in order to facilitate discussion among panelists. Panelists were instructed to refrain from wearing strong perfumes, eating food, chewing gum, drinking juice or soft drinks for 1 h prior to all sessions. Panelists were exposed to a variety of commercial soy protein samples that were similar in attributes to our GSSP and WF samples and were asked to create a list of descriptors for aroma, flavor and mouth-feel. A broad list of attributes was developed, through agreement redundant terms were eliminated, some attributes were combined, and only those attributes found to be most prominent and important in understanding the samples were retained in the final list. The panelists were able to produce and define a concise list of the prominent sensory attributes (Table 3).
In the following sessions, the panelists were presented with references for each attribute until consensus was reached for each reference (Table 3). A 15-cm line scale was used for the descriptive evaluation of the samples. The scale measured from left to right, 0 as minimum and 15 maximum anchored with “weak” and “intense” markers respectively. The references had scores of 15 on this scale, giving the panelists a reference point. Panelists received training on using this 15-point scale on the computer using Compusense five version 4.6 (Compusense Inc., Guelph, ON, Canada).

**Table 3.** List of terms, definitions and references developed by the panelists and used for sensory evaluation

<table>
<thead>
<tr>
<th>Term/Attribute</th>
<th>Definition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fishy</td>
<td>Aroma associated with waste water, fish protein off-aroma</td>
<td>GSSP β-Con-rich fraction 10% (w/v) in water</td>
</tr>
<tr>
<td>Floury</td>
<td>Aroma associated with whole wheat flour</td>
<td>Whole wheat flour 10% (w/v) in water</td>
</tr>
<tr>
<td>Raw beany</td>
<td>Aroma associated with soaked raw soybeans</td>
<td>Raw soaked soybeans 1 Tbsp</td>
</tr>
<tr>
<td>Salty</td>
<td>Taste associated with sea salt or sea water</td>
<td>Sodium chloride 0.4% (w/v) in water</td>
</tr>
<tr>
<td>Floury</td>
<td>Taste associated with whole wheat flour</td>
<td>Whole wheat flour 10% (w/v) in water</td>
</tr>
<tr>
<td>Cooked beany</td>
<td>Taste associated with cooked beans</td>
<td>Canned soybeans 1 Tbsp</td>
</tr>
<tr>
<td>Chalky</td>
<td>Degree of powdery chalk like feeling in mouth</td>
<td>White wheat flour 10% (w/v) in water</td>
</tr>
<tr>
<td>Astringent</td>
<td>Dryness and puckering sensation at back of the tongue</td>
<td>Alum 0.05% (w/v) in water</td>
</tr>
<tr>
<td>Mouthcoating</td>
<td>Degree of film coating on tongue and upper palate of the mouth</td>
<td>Half and Half dairy milk ~30 ml</td>
</tr>
</tbody>
</table>

**Descriptive Analysis**

Sensory evaluation of the test samples was conducted in the sensory evaluation unit in the College of Human Science at Iowa State University. Sensory evaluation was conducted
over two days, one day each for replications 1 and 2. Each panelist evaluated 6 samples a
day. To prepare the samples for evaluation SPI, and gly-rich and $\beta$-con-rich fractions from
WF and GSSP meal were mixed in room-temperature tap water to prepare 6% (w/v)
solutions. The concentration (6% w/v) of the soy protein samples to be evaluated was agreed
upon by the panelists as it was found to be most appropriate to be able to identify and
evaluate the attributes clearly and precisely. About 40 ml of each soy protein sample was
served at room temperature in semi-transparent 2-oz cups with lids. Lids were used to
prevent loss of aroma. Each cup was labeled with a randomly selected three-digit code.
Panelists were provided with unsalted crackers to consume in between sample testing to
prevent any carry-over effects from one sample to the next. They were also provided room-
temperature tap water to drink and rinse between samples.

Soy protein samples and references were presented to panelists seated in individual
booths under white light. A quiet and comfortable environment was maintained. Panelists
were instructed to take three quick sniffs of the sample to evaluate aroma, move the sample
in their mouths and around their tongues before expectorating to evaluate flavor and
mouthfeel; to rinse with and drink water between samples; to eat a cracker; and to wait 5 sec
between before evaluating another sample. SPI and gly-rich and $\beta$-con-rich fractions made
from WF and GSSP meal were evaluated by the panelists for the 9 sensory attributes (Table
3).

Instrumental Analysis

Colors of the samples were measured using the Hunter CIE LAB system. A Hunter
MiniScan XE Plus spectrometer (Hunter Associates Laboratory, Inc., Reston, VA., USA)
was used with the following set parameters; display - absolute, illuminant - D65 (daylight)
and observer - 10º standard. About 50 ml of each sample at 3.6% db protein concentration
was measured in a standardized glass cup over the (1.3-inch/3.302-cm) lens of the spectrometer and the sample was covered with a black cover to prevent outside light interference. This procedure was used to ensure accuracy and consistency when measuring color. Each sample was evaluated three times and means are presented.

**Experimental Design and Statistical Analysis**

Compusense five was used to collect the data from the panelists and obtain sensory evaluation means for each attribute of all samples. A randomized complete block design was used where subjects were treated as blocks. The factorial combination was 2 starting materials and 3 protein products. A 6 x 6 Latin-square design was used to determine the sample serving order for each panelists. The data was analyzed with a mixed model procedure of 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). Comparisons of means was done using Tukey-Kramer HSD test. When a significant interaction between starting material and protein resulted, interaction means were reported. Main-effect means were reported when no significant interactions were found.

**Results and Discussion**

**Composition**

The flour prepared from WFs had slightly higher protein content than the GSSP flour (51 vs 50%, db) due to higher residual oil contents of GSSP meal (Table 1). The total fat contents (1.9-7.2% db) of both flours were significantly higher than their free fat contents (0.9-5.7% db). The free fat and total fat contents of the GSSP flour were much higher than those of the flour prepared from WF (0.9 and 1.9% vs 0.4 and 7.2%, respectively).
Table 1. Compositions of WF and GSSP flours and protein products

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Product</th>
<th>Protein Content (% db)</th>
<th>Free Fat Content (% db)</th>
<th>Total Fat Content (% db)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFs</td>
<td>Flour</td>
<td>51.1</td>
<td>0.9</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>SPI</td>
<td>86.0</td>
<td>0.1</td>
<td>2.1</td>
</tr>
<tr>
<td></td>
<td>Gly-rich</td>
<td>89.6</td>
<td>0.1</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>β-Con-rich</td>
<td>81.0</td>
<td>0.1</td>
<td>3.2</td>
</tr>
<tr>
<td>GSSP Meal</td>
<td>Flour</td>
<td>49.6</td>
<td>5.7</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>SPI</td>
<td>80.3</td>
<td>0.4</td>
<td>6.1</td>
</tr>
<tr>
<td></td>
<td>Gly-rich</td>
<td>86.0</td>
<td>0.6</td>
<td>3.8</td>
</tr>
<tr>
<td></td>
<td>β-Con-rich</td>
<td>71.0</td>
<td>0.8</td>
<td>7.4</td>
</tr>
</tbody>
</table>

*WFs denotes white flakes and GSSP, gas-supported screw pressing.*

The protein contents of SPIs obtained from both WF and GSSP meal were not quite 90% db but were relatively high (80-86%, db); the protein content of the SPI prepared from WFs was higher than SPI prepared from GSSP meal due to the GSSP meal containing substantially more fat. The protein contents of the SPI and the gly-rich and β-con-rich fractions prepared from WFs were higher than those prepared from GSSP soybean meal. We attribute this to the higher PDI of the WFs versus GSSP meal (90 vs 73). GSSP soybean meal had a much higher PDI than typical screw-pressed or extruded-expelled soybean meals, which range from 11 to 18, respectively [21]. Typically, WFs used for the commercial production of SPI have PDI values ~80. Another cause for the difference in protein contents is the higher fat content of the GSSP soybean meal compared to WFs. Some of that free fat became bound to the soy protein during isolation.

**Individual Protein Compositions**

The β-con-rich and gly-rich fractions produced from both WFs and GSSP meal were enriched in β-con and gly, respectively (Table 2); however in previous pilot-plant studies we have achieved greater β-con-to-gly ratios in the β-con-rich fraction (unpublished data). The
amounts of $\beta$-con in each of the three WF proteins were similar to the respective proteins produced from GSSP meals. There were no significant differences in the gly content between WF and GSSP protein products.

**Table 2.** Protein profiles of the soy protein products prepared from white flakes and gas-supported soybean meal (% of total protein)$^a$

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Protein Product</th>
<th>Lipoxygenase</th>
<th>$\beta$-Conglycinin</th>
<th>Glycinin</th>
<th>Others</th>
<th>$\beta$-Con:Gly</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFs</td>
<td>SPI</td>
<td>2$^a$</td>
<td>31$^b$</td>
<td>62$^{bc}$</td>
<td>5$^c$</td>
<td>1:2.0</td>
</tr>
<tr>
<td></td>
<td>Gly-rich</td>
<td>nd</td>
<td>12$^c$</td>
<td>71$^{ba}$</td>
<td>16$^a$</td>
<td>1:5.9</td>
</tr>
<tr>
<td></td>
<td>$\beta$-Con-rich</td>
<td>3$^a$</td>
<td>44$^a$</td>
<td>36$^d$</td>
<td>17$^a$</td>
<td>1:0.8</td>
</tr>
<tr>
<td>GSSP Meal</td>
<td>SPI</td>
<td>nd</td>
<td>25$^b$</td>
<td>56$^c$</td>
<td>19$^a$</td>
<td>1:2.2</td>
</tr>
<tr>
<td></td>
<td>Gly-rich</td>
<td>nd</td>
<td>10$^c$</td>
<td>80$^a$</td>
<td>10$^{bc}$</td>
<td>1:8.0</td>
</tr>
<tr>
<td></td>
<td>$\beta$-Con-rich</td>
<td>4$^a$</td>
<td>47$^a$</td>
<td>29$^d$</td>
<td>14$^{ba}$</td>
<td>1:0.6</td>
</tr>
</tbody>
</table>

$^a n = 3$. Means within a column for a specific protein product followed by different superscripts are significantly different at $p <0.05$. WFs denotes white flakes, GSSP, gas-supported screw pressing nd, non-detectable.

Lipoxygenase was detected in the SPI prepared from WFs but not from GSSP meal in accordance with our previous findings [9,10]. There was no lipoxygenase detected in both gly-rich fractions.

**Descriptive sensory analysis**

Beany sensory attributes are associated with processed soy, panelists were able to detect a raw beany aroma and cooked beany flavor in the protein samples. There were no interactions between the starting materials and the protein products for fishy aroma, floury aroma, raw beany aroma, floury flavor, cooked beany aroma, astringent mouth-feel, and mouthcoating (Tables 4 and 5) indicating that the differences detected in the protein products for these sensory attributes was not due to differences in starting material (WFs and GSSP
soybean meals). There was some interaction between the starting materials and protein products for salty flavor and chalky mouthfeel (Table 6).

Table 4. Main effect means of WF and GSSP soy flours for attributes with no protein interactiona

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Aroma</th>
<th>Flavor</th>
<th>Mouthfeel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fishy</td>
<td>Floury</td>
<td>Raw Beany</td>
</tr>
<tr>
<td>WF</td>
<td>7.13</td>
<td>3.89</td>
<td>2.23</td>
</tr>
<tr>
<td>GSSP</td>
<td>6.77</td>
<td>3.97</td>
<td>2.69</td>
</tr>
<tr>
<td>S/NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

aMain effect means are responses of 12 panelists and 2 replications. WFs denotes white flakes; GSSP, gas-supported screw pressing; S denotes significant effect and NS, no significant effect (p < 0.05).

Table 5. Main effect means of SPI and gly-rich and β-con-rich fractions for attributes with no starting material interactiona

<table>
<thead>
<tr>
<th>Protein Product</th>
<th>Aroma</th>
<th>Flavor</th>
<th>Mouthfeel</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fishy</td>
<td>Floury</td>
<td>Raw Beany</td>
</tr>
<tr>
<td>SPI</td>
<td>1.98</td>
<td>5.16a</td>
<td>3.28a</td>
</tr>
<tr>
<td>Gly-rich</td>
<td>9.76a</td>
<td>3.23b</td>
<td>1.99b</td>
</tr>
<tr>
<td>β-Con-rich</td>
<td>9.12a</td>
<td>3.41b</td>
<td>2.11b</td>
</tr>
</tbody>
</table>

aMain effect means are responses of 12 panelists and 2 replications. Means within a column followed by different superscripts are significantly different at p < 0.05.

Difference in the extraction process did not affect the sensory properties (Table 4), except for differences in mouthcoating. Protein products prepared from GSSP meal had significantly greater mouthcoating than those produced from WFs, which we attribute to the higher fat contents (Table 1). Samoto et al. [22] found that the off-flavors in SPI prepared from WFs were due to the polar lipids, which bind to oil-body-associated protein. We hypothesized that protein products prepared from the GSSP flour would be different from those prepared from WFs in sensory attributes due to their higher fat contents, but this was not observed. This is a fortunate finding, showing that the higher levels of fat do not greatly
affect sensory properties. One possible explanation for lipid-derived off-flavors not being detected in the GSSP protein products could be that the lipoxygenase was deactivated by heat during GSSP and any residual active lipoxygenase was washed away during the protein isolation and fractionation process.

Table 6. Sensory evaluation of protein products produced from WFs and GSSP meal for attributes with starting material interactiona

<table>
<thead>
<tr>
<th>Starting Material</th>
<th>Protein Product</th>
<th>Salty Flavor</th>
<th>Chalky Mouthfeel</th>
</tr>
</thead>
<tbody>
<tr>
<td>WFs</td>
<td>SPI</td>
<td>4.61b</td>
<td>3.97bc</td>
</tr>
<tr>
<td></td>
<td>Gly-rich</td>
<td>2.80c</td>
<td>4.65ab</td>
</tr>
<tr>
<td></td>
<td>β-Con-rich</td>
<td>3.77bc</td>
<td>3.22c</td>
</tr>
<tr>
<td>GSSP Meal</td>
<td>SPI</td>
<td>3.30bc</td>
<td>3.27c</td>
</tr>
<tr>
<td></td>
<td>Gly-rich</td>
<td>4.76b</td>
<td>5.32a</td>
</tr>
<tr>
<td></td>
<td>β-Con-rich</td>
<td>6.79a</td>
<td>4.54ab</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>1.53</td>
<td>1.12</td>
</tr>
</tbody>
</table>

*Means within a column followed by different superscripts are significantly different at p < 0.05. WFs denotes white flakes and GSSP, gas-supported screw pressing. 0 = minimum intensity, 15 = maximum intensity score.

Evaluation of the three protein products independent of starting material indicates that SPI is different from the gly-rich and β-con-rich protein fractions in all sensory properties, except for cooked beany flavor and degree of mouthcoating (Table 5). The uniform cooked flavor may be due to the jet-cooking process that was used in producing all protein products. Degree of mouthcoating was not affected by the protein isolation/fractionation process used; hence, no difference in that attribute.

All of our protein products had fishy aromas. Unfortunately, the gly-rich and β-con-rich fractions had significantly stronger fishy aroma than the SPIs. This could be due to the presence of volatile amines. Arai et al. [23] investigated the flavor contribution of volatile amines present in raw soybeans and found ammonia, monomethylamine, dimethylamine, piperidine and cadaverine to have odors resembling dried fish.
Floury aromas and flavors were detected in all protein products, however, they were significantly more intense in the SPIs. Similar floury sensory attributes have also been found in commercial SPIs by a descriptive sensory panel [24].

Green beany aroma is common in soybeans and its products, and is attributed to the presence of volatile neutral compounds including isopentanol, $n$-hexanol and $n$-heptanol [25]. The raw beany aroma, floury aroma and floury flavor in the SPIs were more intense compared to the gly-rich and $\beta$-con-rich protein fractions.

The SPIs had significantly less astringent mouthfeel compared to the gly-rich and $\beta$-con-rich fractions. Astringent sensation is caused by the interaction of phenolic compounds in foods with saliva proteins in the mouth [26]. Arai et al. [27] identified seven phenolic compounds in soybeans, which they indicated could be responsible for the astringent mouthfeel in soy. They suggested that these phenolic compounds are present in soy protein products because they are not affected by the hexane-extraction process since they do not dissolve in hexane and are heat stable.

The GSSP $\beta$-con-rich fraction had significantly greater salty flavor compared to all other protein fractions. We are unable to explain the differences observed for this attribute. WF $\beta$-con-rich fraction and GSSP SPI had significantly lower chalky mouthfeel; this was surprising since there were no differences in particle size of all protein samples.

**Color**

For color analyses, sample dispersions were evaluated at the 3.6% db protein concentration of soymilk [28] so that we could use soymilk as a comparative reference (L=83.00, a=1.45, b=10.28) [29]. Since there was no interaction between starting materials and proteins, we evaluated the differences attributable to the oil-extraction process and protein extraction individually.
The dispersions of the protein products prepared from WFs and GSSP meal had similar L* and a* values, but had significantly different b* values (Table 7). Indicating that they were similar in lightness and green color, but the GSSP protein products had a significantly greater yellow color. Compared to the reference soymilk, the dispersions of the protein products were darker and greener. The yellowness of the dispersions of GSSP protein products was the greatest, followed by the reference soymilk and then the dispersions of WFs products. The greater yellowness of the dispersions of GSSP protein products were attributed to the higher fat contents of the protein products prepared from GSSP meal.

Among the protein products, SPI had a significantly lower L* value, there were no differences in the a* and b* values (Table 8). That is the SPI dispersions were significantly
darker than the gly-rich and β-con-rich fractions, with no differences in green and yellow colors among dispersions of these protein products.

Conclusions

Protein products prepared from GSSP meal can be used as substitutions for protein products prepared from WFs in foods. Protein products prepared from GSSP meal have greater mouthcoating properties compared to protein products prepared from WFs. The type of protein significantly affected sensory properties. SPIs had less fishy aroma and astringency than the gly-rich and β-con-rich protein fractions. On the other hand, the gly-rich and β-con-rich protein fractions had less floury aroma, raw beany aroma and floury flavor. Further investigation is warranted to identify the compounds responsible for these sensory attributes.

Acknowledgments

The authors gratefully acknowledge the support of the Grow Iowa Values Fund, the Iowa Agricultural and Home Economics Experiment Station, Crown Iron Works and SafeSoy Technologies.

References


CHAPTER 5. GENERAL CONCLUSIONS

General Discussion

The goal of our research was to evaluate soy protein isolates (SPI) made from gas-supported screw pressed (GSSP) soybean meal in comparison to SPI prepared from white flakes (WFs) by studying compositional, functional, and sensory characteristics. We used hydrogen peroxide as a preservation treatment in comparison to jet-cooking and investigated their effects on soy protein functionality. We also evaluated glycinin-rich and β-conglycinin-rich fraction prepared from GSSP meal for their sensory properties in comparison to SPI produced from GSSP meal and WFs.

Our laboratory study demonstrated that it is possible to produce SPIs from GSSP meal with >90% protein content. Despite the high fat content of the GSSP meal compared to WFs, greater protein yields were obtained from the GSSP meal. SPI prepared from GSSP meal had some functional advantages over SPI prepared from WFs – better WHC, higher viscosity, better emulsification properties and fat-binding properties. SPI prepared from GSSP meal had similar to slightly poorer water-solubility and foaming properties.

Summary of GSSP properties from the laboratory study

<table>
<thead>
<tr>
<th>Property</th>
<th>GSSP vs WF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein dispersibility index</td>
<td>Lower</td>
</tr>
<tr>
<td>Yields</td>
<td>Higher</td>
</tr>
<tr>
<td>Lipoxygenase</td>
<td>Nd</td>
</tr>
<tr>
<td>Thermal enthalpy</td>
<td>Higher</td>
</tr>
<tr>
<td>Solubility</td>
<td>Lower</td>
</tr>
<tr>
<td>Water-holding capacity</td>
<td>Higher</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Higher</td>
</tr>
<tr>
<td>Emulsification</td>
<td>Higher</td>
</tr>
<tr>
<td>Fat-binding capacity</td>
<td>Higher</td>
</tr>
<tr>
<td>Foaming</td>
<td>Lower</td>
</tr>
</tbody>
</table>
SPI prepared from GSSP meal was successfully produced on large scale (20 Kg meal) in the pilot plant, where it was spray-dried similar to industry practice. Pilot-plant production of SPI on a large scale resulted in altered functional properties compared to our laboratory study (100g meal). The SPI prepared from GSSP meal had solubility, water-holding capacity, viscosity, fat-binding capacity and emulsification properties similar to SPI prepared from WFs. The SPI prepared from GSSP meal had poorer foaming properties, which we associate with its high fat content. H$_2$O$_2$-treated SPIs had similar to better functionality than jet-cooked SPIs; making it a viable alternative to jet-cooking for preserving SPI. Not only would this result in an SPI with less denatured glycinin and β-conglycinin but would eliminate a heating step in the processing of SPI.

<table>
<thead>
<tr>
<th>Functional Property</th>
<th>GSSP vs WF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein dispersibility index</td>
<td>Lower</td>
</tr>
<tr>
<td>Yields</td>
<td>Lower</td>
</tr>
<tr>
<td>Lipoxygenase</td>
<td>Nd</td>
</tr>
<tr>
<td>Thermal behavior</td>
<td>Similar</td>
</tr>
<tr>
<td>Solubility</td>
<td>Slightly lower</td>
</tr>
<tr>
<td>Water-holding capacity</td>
<td>Similar</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Similar</td>
</tr>
<tr>
<td>Emulsification</td>
<td>Similar</td>
</tr>
<tr>
<td>Fat-binding capacity</td>
<td>Similar</td>
</tr>
<tr>
<td>Foaming</td>
<td>Lower FSI &amp; F rate</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Functional Property</th>
<th>H$_2$O$_2$ vs Jet cooking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thermal enthalpy</td>
<td>Less denaturation</td>
</tr>
<tr>
<td>Solubility</td>
<td>Higher</td>
</tr>
<tr>
<td>Water-holding capacity</td>
<td>Lower</td>
</tr>
<tr>
<td>Viscosity</td>
<td>Lower</td>
</tr>
<tr>
<td>Emulsification</td>
<td>Higher</td>
</tr>
<tr>
<td>Fat-binding capacity</td>
<td>Similar</td>
</tr>
<tr>
<td>Foaming</td>
<td>Similar</td>
</tr>
</tbody>
</table>

Summary of GSSP properties from the pilot-plant study

Summary of effects of H$_2$O$_2$ preservation method from the pilot-plant study
It is important to not only have a functional protein but it also needs to taste good in food. Our descriptive sensory panel study revealed that protein products produced from GSSP meal were similar in sensory attributes to protein products produced from WFs. Protein products produced from GSSP meal had greater mouthcoating, probably due to their higher fat contents. They were also similar in lightness and green color, but protein products produced from GSSP meal were more yellow than protein products produced from WFs. We were also able to fractionate proteins in GSSP meal to produce glycينin-rich and $\beta$-conglycinin-rich fractions. When comparing SPI and glycínin-rich and $\beta$-conglycinin-rich fraction, the fractions had significantly stronger fishy aroma, astringent mouthfeel, lower floury aroma, raw beany aroma and floury flavor than SPI.

These studies support our hypothesis that GSSP soybean meal can be used to produce high quality SPIs with similar or better properties compared to protein products prepared from white flakes (WF). Fractionated soy protein needs to deliver some advantages that we have not yet identified to overcome disadvantages of increased cost and poorer flavor.

**Recommendations for Future Research**

Since GSSP is a new process and the protein products made using this process are also new, a lot more research is needed to completely understand its full potential. Since it can be used to produce organic soy protein products, it has huge potential market demand.

In order to gain a better understanding the functionality of these new protein products, it might be of value to study the proteins in different food systems such as beverages, baked foods, meats and dairy analogs. It would also be valuable to be able to
identify the compounds responsible for the unique sensory attributes using gas-chromatography.

No lipoxygenase detected in the GSSP produced proteins needs further investigation. From electrophoretic gels we also see that there is more subunit separation in the protein products prepared from GSSP meal compared to those prepared from WFs.

**Acknowledgements**

I would like to acknowledge my major professor Dr. Lawrence Johnson for his guidance and support throughout my masters program at Iowa State University. I would like to thank Dr. Cheryll Reitmeier for her guidance through my sensory study and for being on my committee. Lastly would also like to thank Dr. Charles Glatz for being on my committee.

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I thank the Center for Crops Utilization Research center and its staff for all the help they provided me. And Iowa State University and the Food Science and Human Nutrition Department for providing me with the opportunity to pursue my education and fulfill my dreams.

Last but not the least I would like to thank my family and friends for all their love and support.
APPENDIX A. SENSORY PANEL QUESTIONNAIRE

Demographic information

Name: ___________________________                                        Gender: ___________
E-mail: ____________________________                                         Age group:  18-24 □
Phone number:____________________                                                             25-29 □
                                 □  30-39 □
                                 □  40-49 □
                                 □  50+   □

Health

1. Do you any of the following?
   □  Diabetes
   □  Food allergies, if yes to what? _________
   □  Sinusitis/ Chronic colds
2. Do you take any medications which will affect your senses, especially taste and smell?
   _____ if yes, please explain __________________________________________

Food Habits

1. Are you currently on a restricted diet? _____ If yes, please explain ________________
2. What are some of your favorite foods?_______________________________________
3. What foods do you not like to eat?  _________________________________________
4. Is your ability to distinguish smells and tastes?
   Smell        Taste
   Better than Average  □        □
   Average            □        □
   Worse than Average □        □

Flavor Quiz

1. If a recipe calls for pepper and there is none available, what would you substitute?_______
2. What are some other foods that taste like yogurt? _______________________________
3. How would you describe the difference between flavor and aroma?
   ___________________________________________________________________________
   ___________________________________________________________________________
4. What are the best one or two words to describe milk?_____________________________
5. Describe some noticeable flavors in:
   Chocolate ________________________________________________________________
   Bread ________________________________________________________________
   Cola _______________________________________________________________

**** Please refrain from eating food, chewing gum, drinking juice or soda 1 hour
   prior to all sessions. Also please refrain from wearing strong perfumes. ****
   Thank you.
APPENDIX B. BASIC SENSORY TESTING

Name: _____________________                                          Date: ___________________

***Please taste all of the codes samples using the following protocol; Rinse your mouth with water, sip the sample and move it in your mouth and around your tongue, then expectorate the sample. Take a sip of water and wait 5 seconds before tasting the next sample. Crackers may also be eaten to cleanse your palate.***

Test 1

Please taste the 5 samples using the protocol above. Select which descriptor you think is appropriate for each sample and write it in the space below.
Descriptor options: Sweet, Sour, Bitter, Salty and Astringent.

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Descriptor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Test 2

Please taste the samples in each set using the protocol above from left to right. In each set two samples are identical and one is different. Circle the sample that is different in each set.

Set 1 :      _______                  _______                _______
Set 2 :      _______                  _______                _______
Set 3 :      _______                  _______                _______
Set 4 :      _______                  _______                _______
**Test 3**

Please taste the samples using the protocol above. Rank the coded samples in each set in ascending order for each attribute.

<table>
<thead>
<tr>
<th>Sample code</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Least sweet</td>
<td>________</td>
<td>________</td>
</tr>
<tr>
<td>Most sweet</td>
<td>________</td>
<td></td>
</tr>
<tr>
<td>B. Least sour</td>
<td>________</td>
<td></td>
</tr>
<tr>
<td>Most sour</td>
<td>________</td>
<td></td>
</tr>
<tr>
<td>C. Least salty</td>
<td>________</td>
<td></td>
</tr>
<tr>
<td>Most salty</td>
<td>________</td>
<td></td>
</tr>
<tr>
<td>D. Least bitter</td>
<td>________</td>
<td></td>
</tr>
<tr>
<td>Most bitter</td>
<td>________</td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX C. SENSORY SCORE SHEET FOR SOY PROTEIN SAMPLES

Panelist Code: _______                                        Date: ______________

Sample evaluating protocol:

Take three quick sniffs of the sample and describe its aroma. Rinse your mouth with water; sip the sample and move it in your mouth and around your tongue, then expectorate the sample. Take a sip of water, eat some cracker and wait 5 seconds before evaluating the next sample.

Directions: Place a perpendicular line, labeled with the number of the sample, on the horizontal line to indicate your assessment of the soy protein samples provided.

AROMA

FISHY AROMA – Waste water, fish protein off-flavor.  Reference standard # 1

FLOURY AROMA. - Whole wheat flour in water. Reference standard # 2

RAW BEANY AROMA – Soaked raw soybeans. Reference standard # 3
FLAVOR

**SALTY FLAVOR** - Table salt in water. Reference standard # 4

**FLOURY FLAVOR** - Whole wheat flour in water. Reference standard # 2

**COOKED BEANY FLAVOR** - Canned soybeans. Reference standard # 5

MOUTHFEEL

**CHALKY MOUTHFEEL** – Powdery chalk like feeling in the mouth. Reference standard # 6
**ASTRINGENT MOUTHFEEL** – Puckering & dryness at the back of the tongue. Reference std. # 7

**MOUTHCOATING** – Film coating on the tongue & upper palate. Reference standard # 8