J Amer Oil Chem Soc (2007) 84:259–268
DOI 10.1007/s11746-007-1035-7

ORIGINAL PAPER

Effects of Extraction Temperature and Preservation Method on Functionality of Soy Protein

Nicolas A. Deak · Lawrence A. Johnson

Received: 30 May 2006 / Accepted: 18 December 2006 / Published online: 24 January 2007

Abstract The effects of extraction temperature and preservation method on the functional properties of soy protein isolate (SPI) were determined. Four extraction temperatures (25, 40, 60, and 80 °C) were used to produce SPI and yields of solids and protein contents were determined. Three preservation methods were also tested (spray-drying, freeze-drying, and freezing–thawing) and compared to fresh (undried) samples for each extraction temperature. No differences in yields of solids and protein were observed among SPIs extracted at 25, 40, and 60 °C; however, SPI extracted at 80 °C yielded significantly less solids and protein. Extraction temperature significantly affected SPI functionality. As extraction temperature increased, solubility and emulsification capacity decreased; surface hydrophobicities, emulsification activities and stabilities, and dynamic viscosities increased; and foaming properties improved. Preservation method also significantly affected SPI functionality. Drying method did not affect the denaturation enthalpies of SPIs, but spray-dried SPIs had higher solubilities, surface hydrophobicities, and emulsification stabilities, and lower viscosities, emulsification activities and rates of foaming than freeze-dried SPI exhibited. Emulsification and foaming capacities and foaming stabilities were similar for both methods of drying. There was significant interaction between extraction temperature and preservation method for all functional properties except emulsification capacity.

N. A. Deak · L. A. Johnson (✉)
Department of Food Science and Human Nutrition,
Center for Crops Utilization Research,
Iowa State University, 1041 Food Science Building,
Ames, IA 50011-1061, USA
e-mail: ljohnson@iastate.edu

Keywords Soy protein isolate · Soybeans · Freeze-drying · Protein · Spray-drying · Solubility · Surface hydrophobicity · Emulsification · Foaming · Dynamic viscosity · Functionality

Introduction

A substantial body of research has prompted the Food and Drug Administration to approve a cholesterol-lowering health claim for soy protein indicating that daily consumption of 25 g soy protein (6.5 g of soy protein per serving) may lower LDL cholesterol in individuals who have high cholesterol and who also adhere to a low-fat diet [1]. Soy protein isolates (SPIs) are the most highly refined soy protein products used as food ingredients, and the use of SPI has been increasing steadily for the last two decades [2]. SPI is prepared from defatted soy meal and contains more than 90% (dry basis) protein. SPI is used as an ingredient in high-protein foods, especially in dairy products, nutritional supplements, meats, infant formulae, nutritional beverages, soups, sauces, and snacks. SPI utilization is based on its wide range of highly desirable functional properties, such as solubility, hydrophobicity, emulsification, foaming, fat and water absorption, gelling, and viscosity control. These important functional properties affect the suitability of SPIs for various applications and, as a result, their value in the marketplace. Stabilization during the storage and distribution of SPIs is critical for food safety, but also for preserving good functionality. Generally, long-term protein preservation is accomplished by drying to less than 8% moisture.

Heat denaturation is a major factor influencing protein functionality [3]. Usually, SPI is produced by extracting defatted soy flakes/flour with alkali at temperatures of between 20 and 80 °C (60 °C being usual). Freeze-drying uses low temperatures for extended periods [4], while spray-drying utilizes high temperatures for short periods of time [5]. Drying affects protein functionality because it usually involves the use of high temperatures, and proteins are thermally unstable and denature [4]. For this reason, we hypothesized that both extraction temperature and drying method significantly affect SPI functionality. Two methods commonly utilized to obtain acceptable long-term storage stability of SPIs are freeze-drying and spray-drying.

Freeze-drying is widely used in scientific research. The process involves removing water from frozen protein dispersions by sublimation under vacuum followed by controlled heating to moderate temperatures to remove the remaining water; higher temperatures are avoided until substantial water is evaporated and water activity is reduced. Residual moisture levels are often less than 1%. Freeze-drying is believed to be the best method to stabilize protein functionality [4].

On the other hand, spray-drying is the primary method used by the food industry for commercial production, especially to produce milk powder, dairy products, and food protein ingredients such as SPI. Spray-drying rapidly dries solutions or slurries to particulate forms by atomizing the liquid in a heated chamber. Spray-drying typically consists of preconcentrating the liquid (for more economic operation, since evaporation is expensive), atomizing (creation of droplets), drying in a stream of hot, dry gas (usually air), separating the powder from the moist gas, cooling, and packaging.

Surprisingly, little has been published about the effects of extraction temperature and preservation method on the functional properties of SPI. Boatright and Hettiarachchy [6] found that spray-dried SPI had higher solubilities than did freeze-dried SPI. The objectives of the present study were to evaluate the effects of extraction temperature and preservation method on the yield, composition and functional properties of SPI.

Experimental Procedures

Materials

SPIs were produced from air-desolventized, hexane-defatted white flakes (IA 2020 variety, 1999 harvest) extracted in the pilot plant of the Center for Crops Utilization Research by using an extractor-simulator (French Oil Mill Machinery Co., Piqua, OH, USA). The defatted flakes were milled with a Krups grinder (Distrito Federal, Mexico) to achieve 100% of the material passing through a 50-mesh screen by using small quantities (10 g) to preserve the native protein state. The protein content of the flour was 57.3% on a dry-weight basis with a protein dispersibility index (PDI) of 93.8 as determined by Silliker Laboratories (Minnetonka, MN, USA). The flour was stored in sealed containers at 4 °C until used.

SPI Preparation

SPI was prepared as shown in Fig. 1. About 200 g of defatted soy flour was extracted with deionized water at a 10:1 water-to-flour ratio, the pH was adjusted to 8.5 with 2 N NaOH, and the resulting slurry was stirred for 30 min. Four different extraction temperatures (25, 40, 60, and 80 °C) were evaluated in triplicate. After centrifuging at 14,300x*g* for 30 min, the protein extract was decanted, and the amount of insoluble fiber residue was determined and sampled for proximate composition. The protein extract was cooled to 4 °C, adjusted to pH 4.5 with 2 N HCl, and centrifuged as described above. Protein curd was obtained as a precipitate, and the amount of supernatant (whey) was determined and sampled for proximate composition. The curd was redissolved in deionized water, and sufficient 2 N NaOH was added to achieve pH 7.0 with approximately 10% solids. All extraction trials were replicated three times.

Preservation

Four samples were taken from each SPI slurry. One sample was analyzed fresh (within 24 h of preparation), another sample was analyzed after freezing for at least two days and then thawing, a third sample was analyzed after freeze-drying, and the last sample was analyzed after spray-drying. For freezing and freeze-drying, the samples were frozen at –80 °C for at least 48 h. One frozen sample was then placed in a Virtis Ultra 35 (Gardiner, NY, USA) freeze-dryer with shelves cooled at –20 °C. High vacuum was then applied while the temperature was held constant (–20 °C) until the vacuum dropped to 100 mTorr. Secondary drying was achieved by heating the freeze-dryer shelves to 26 °C at high vacuum. The complete freeze-drying cycle took 120 h.

For spray-drying, the protein slurries were fed at about 7 ml/min and 25 °C to a Yamato Pulvis spray-dryer (Model GB-21, Yamato Scientific Co. Ltd., Yamanashi, Japan). The air-inlet temperature was

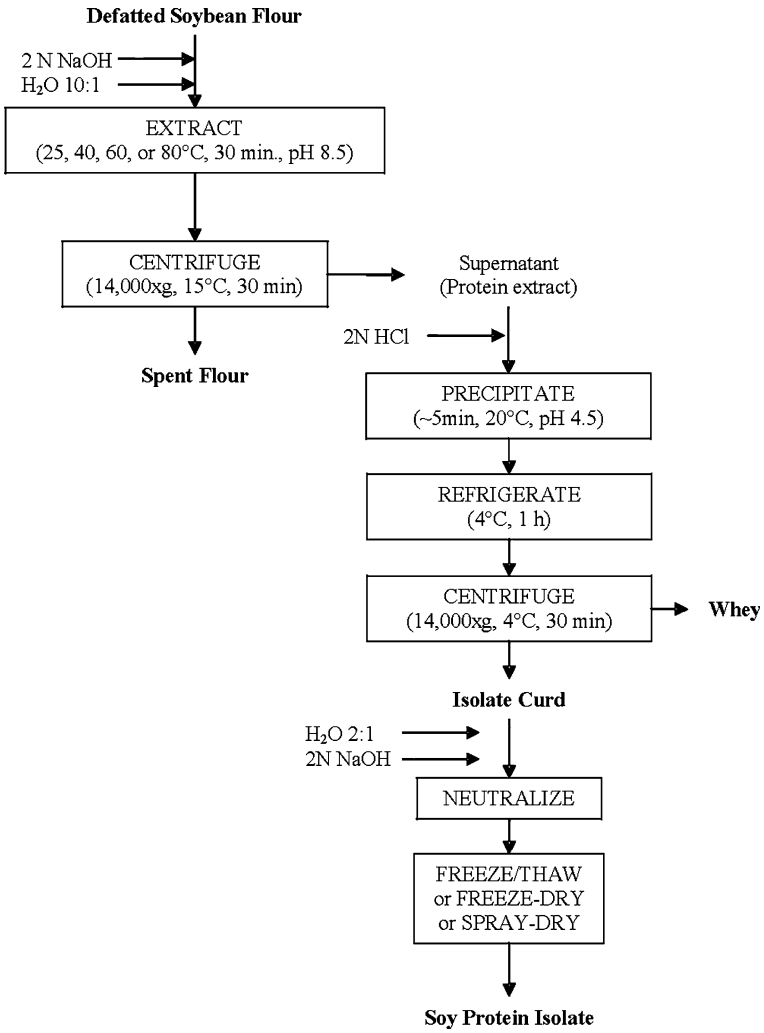

Fig. 1 Soy protein isolation procedure

160 °C, the air-outlet temperature was 80 °C, and the pulverizer air flow was set at 2.5 kg force/cm^2. All preservation treatments were replicated three times for each extraction temperature.

Proximate Analyses and Mass Balances

Nitrogen contents of the soy flour, isolated protein products and byproduct streams were measured by using the combustion or Dumas method [7] with a Rapid NIII Analyzer (Elementar Americas, Inc., Mt. Laurel, NJ, USA). The nitrogen values were converted to Kjeldahl nitrogen by using the conversion formula of Jung et al. [8]. All measurements were determined at least three times and means were reported. The conversion factor used to estimate protein content was N×6.25. Moisture was determined by oven drying for 3 h at 130 °C [9]. Mass balances of solids and protein were determined for all SPI treatments. Analyses were replicated in triplicate and means reported.

Thermal Behavior

Thermal behavior of the isolated proteins was assessed using differential scanning calorimetry (DSC). Sample dispersions (15–20 mg) of 10% (w/w, dry basis) protein were hermetically sealed in aluminum pans. A sealed, empty pan was used as reference. The samples were heated from 25 to 120 °C at 10 °C/min using an SII Exstar 6000 (Seiko Instrument, Inc., Tokyo, Japan). All samples were analyzed at least three times and means were reported.

Solubility

Solubility was evaluated according to the methods of Rickert et al. [10]. The samples were tested at pH 7.0. Solubility was calculated as: % solubility = [amount of protein in supernatant/amount of initial protein in the sample] × 100. All samples were analyzed at least three times and means were reported.

Surface Hydrophobicity

Surface hydrophobicity was measured by using the methods of Wu et al. [11] with modifications. Protein dispersions were prepared as in the solubility test and aliquots of the soluble protein (supernatant) were serially diluted to obtain 6.25–100 μg/ml protein with 0.1 M sodium phosphate buffer (pH 7.0) as diluent. To 3-ml aliquots of each dilution, 40 μl of 1-anilino-8-naphthalene sulfonic acid magnesium salt monohydrate (ANS, ICN Biomedicals, Inc., Aurora, OH, USA) (8.0 mM in 0.01 M phosphate buffer, pH 7.0) was dispersed. Fluorescence intensity units (FIU) were measured with a Turner Quantech spectrophotometer (Barnstead Thermolyne, Dubuque, IA, USA) using 440 nm (excitation) and 535 (emission) filters. FIU were standardized using a solution of 40 μl of ANS in 3 ml of phosphate buffer as the zero point and 15 μl of ANS in 3 ml of methanol assigned an arbitrary value of 80 FIU. FIUs were plotted against percentages of protein concentration. The slope of the regression line was reported as the surface hydrophobicity. Samples were run in triplicate and means were reported.

Emulsification Properties

Emulsification capacity was measured according to the methods of Bian et al. [12] with modifications. Twenty-five milliliters of a 2% (w/w, dry basis) sample dispersion adjusted to pH 7.0 with 2 N HCl or NaOH was transferred to a 400-ml plastic beaker. Soybean oil, dyed with approximately 4 ppm Sudan Red 7B (Sigma, St. Louis, MO, USA), was continuously blended into the dispersion at 37 ml/min flow rate using a Bamix wand mixer (ESGE AG Model 120, Mettlen, Switzerland) at the low setting until phase inversion was observed. Emulsification capacity (g oil/g sample) was calculated as g of oil used to cause inversion multiplied by 2. Emulsification activity and emulsification stability index were measured according to methods of Rickert et al. [10]. All analyses were replicated at least three times and means were reported.

Foaming Properties

Foaming properties were measured according to the methods of Sorgentini et al. [13], with modifications developed by Rickert et al. [10]. A 0.5% (w/w, dry basis) sample dispersion was prepared and the pH adjusted to 7.0. A 95-ml aliquot was loaded into a custom-designed glass column (58.5 cm × 2 cm) fitted with coarse-fritted glass at the bottom, and N_2 gas was purged through the sample at 100 ml/min flow rate. Time for the foam to reach 300-ml volume, time for one-half of the liquid incorporated into the foam to drain back, and volume of the liquid incorporated into the foam were measured. Three parameters were calculated:

$$\text{Foaming capacity (FC)} = V_f/(f_r \times t_f)$$
$$K \text{ (specific rate constant of drainage)} = 1/(V_{max} \times t_{1/2})$$
$$V_i \text{ (rate of liquid conversion to foam)} = V_{max}/t_f$$

where V_f = a fixed volume of 300 ml, f_r = the flow rate of the gas, t_f = time to reach V_f, V_{max} = volume of liquid incorporated into foam, and $t_{1/2}$ = time to drain one-half of the liquid incorporated into the foam. Samples were run in triplicate and means were reported.

Dynamic Viscosity

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

Statistical Analysis

The data were analyzed according to the split-plot experimental design by analysis of variance (ANOVA) and the mixed model by the SAS system (version 8.2, SAS Institute Inc., Cary, NC, USA). Least significant differences (LSD) were calculated at the 5% level to compare whole-plot and split-plot treatment means for each response variable.

Results and Discussion

Yields and Protein Contents

SPIs extracted at 25, 40, and 60 °C gave similar yields of solids (~42%) and yields of proteins (~72%), and had similar protein contents (~92%) (Table 1). SPIs extracted at 80 °C had lower yields of solids (~39%), yields of protein (~63%), and protein contents (~88%) due to protein denaturation during extraction and, as a consequence, loss of protein solubility.

Table 1 Solids yields, protein yields and protein contents of soy protein isolate before preservation

Extraction temperature (°C)	Solids yield (%)	Protein yield (%)	Protein content (%, $N = 6.25$)
25	42.62 a	71.88 a	91.07 a
40	41.64 a	71.59 a	92.83 a
60	42.70 a	71.95 a	91.23 a
80	39.11 b	63.27 b	88.21 b
LSD	2.50	1.99	1.86

Means in the same column followed by different letters are significantly different ($p < 0.05$, $N = 3$). *LSD* denotes least significant difference

Thermal Properties

There were no significant differences among denaturation onset temperatures or denaturation peak temperatures for any of the SPIs prepared at extraction temperatures of 25, 40, and 60 °C and by any of the preservation methods (denaturation onset temperatures and peak temperatures were 67.1 ± 1.0 and 73.9 ± 0.6 °C for β-conglycinin, and 83.0 ± 0.9 and 91.1 ± 0.5 °C for glycinin, respectively). The SPI extracted at 80 °C had no thermally active native structure remaining. Lower extraction temperatures, however, significantly affected denaturation enthalpies. SPIs extracted at 60 °C had reduced denaturation enthalpies (greatly reduced β-conglycinin enthalpy and slightly reduced glycinin enthalpy). These reduced enthalpies were probably caused by the combination of temperature and alkali (pH 8.5 during the extraction step), since onset denaturation temperatures at pH 7.0 were above 60 and 80 °C for β-conglycinin and glycinin, respectively, the extraction temperatures used for samples where significant enthalpy reductions were observed.

Reduced denaturation enthalpies for both β-conglycinin and glycinin were observed after both methods of drying at all extraction temperatures (Table 2). There were no significant differences between the denaturation enthalpies of the freeze-dried and the spray-dried samples extracted at the same temperature. These findings indicated that both drying methods denature soy proteins to the same extent despite major differences in time/temperature exposure. Denaturation enthalpy was significantly reduced by freezing and thawing, except for β-conglycinin extracted at 60 °C; however, this reduction could not account for the total loss of enthalpy observed in freeze-dried samples. Significant denaturation must have occurred during the sublimation phase of freeze-drying.

Significant interaction was observed between extraction temperature and preservation method. For denaturation enthalpy of β-conglycinin, there was weak interaction with an F-value of 2.75 and a p-value of 0.044. For the glycinin component, there was stronger interaction evidence with an F-value of 21.06 and a p-value of <0.0001. The error degrees of freedom for all cases were 18. The interaction between extraction temperature and preservation method was probably due to the denaturation caused by the preservation method depending on the previous thermal history of the protein. The lower significance level for the β-conglycinin component was probably due to this protein being more susceptible to denaturation and, as a consequence, denatured to a similar extent regardless of the methods of extraction and preservation. On the other hand, the glycinin component was less susceptible to denaturation and was partially denatured to different extents depending on the extraction temperature and preservation method. This significant interaction means that preservation method and extraction temperature are nonadditive factors affecting denaturation and, hence, each preservation method must be compared individually at each extraction temperature.

Table 2 Effects of extraction temperature and preservation method on denaturation enthalpies (ΔH, mJ/mg) of soy protein isolate

Treatment	Extraction temperature (°C)		
	25	40	60
Denaturation enthalpy of β-conglycinin[1]			
Fresh	2.58^{a} a	2.63^{a} a	0.70^{a} b
Frozen/thawed	2.23^{b} a	2.29^{b} a	$0.65^{a,b}$ b
Freeze-dried	2.07^{b} a	2.25^{b} a	$0.52^{b,c}$ b
Spray-dried	2.16^{b} a	2.20^{b} a	0.44^{c} b
Denaturation enthalpy of glycinin[2]			
Fresh	7.79^{a} a	7.04^{a} b	6.72^{a} c
Frozen/thawed	7.51^{b} a	6.83^{b} b	6.49^{b} c
Freeze-dried	6.51^{c} a	6.53^{c} a	6.21^{c} b
Spray-dried	6.50^{c} a	6.53^{c} a	6.26^{c} b

LSD denotes least significant difference, $p < 0.05$, $N = 3$

[1] LSD for means within the same row is 0.14, means followed by different full case letters within a row are significantly different. LSD for means within the same column is 0.15, means followed by different lower case superscript letters within a column are significantly different

[2] LSD for means within the same row is 0.18, means followed by different full case letters within a row are significantly different. LSD for means within the same column is 0.16, means followed by different lower case superscript letters within a column are significantly different

Solubility

Solubility is an important functional property of protein ingredients, since protein solubility affects most other functional properties [14]. The highest solubili-

ties were for SPIs extracted at 25, 40, and 60 °C, followed by SPI extracted at 80 °C (Table 3). No significant difference in solubility was observed among preservation methods when the SPI was extracted at 40 and 60 °C. The solubility of the SPI extracted at 25 °C was affected more by the preservation method than were the SPIs extracted at 40 and 60 °C, probably because the products had significantly higher amounts of native protein.

Freeze-dried SPIs were significantly less soluble than spray-dried SPIs, confirming previous observations of Boatright and Hettiarachchy [6]. Freeze-dried SPIs were easier to disperse in water than spray-dried SPIs, probably due to the electrostatic charges on the particle surfaces of spray-dried SPI.

There was significant interaction between extraction temperature and preservation method for solubility of the SPI. The F-value for the interaction was 38.05 with a corresponding p-value of <0.0001, and the error degrees of freedom were 24. Protein extraction at 80 °C caused large losses in solubility, probably because of a high degree of aggregation.

The formation of different sizes of aggregates would partially explain the effects of preservation method on solubility. In general, the solubility of SPI decreased when freeze-thawed. This behavior could be caused by the increased size of aggregates formed during acid precipitation due to prior temperature treatment. This model would also explain why the freeze-dried SPIs at similar degrees of denaturation had lower solubilities than did spray-dried SPIs. Evidently, the degree of aggregation was dependent on the original amount of denatured protein present in the SPI, which in turn was highly dependent on extraction temperature. The degree of aggregation depends on the previous thermal treatment and, as a consequence, the size of the aggregates formed in SPI dispersions affects solubility [15–17]. The formation of soluble and insoluble aggregates of SPI on heating has been widely reported, as has the nature of these aggregates and the interaction of different soy protein components [13, 16, 18–20].

Table 3 Effects of extraction temperatures and preservation method on protein solubility (%) of soy protein isolate at pH 7.0

Treatment	Extraction temperature (°C)			
	25	40	60	80
Fresh	94.9^{a} a	94.9^{a} a	93.4^{a} a	83.5^{a} b
Frozen/thawed	89.4^{c} b	92.0^{b} a	$92.0^{a,b}$ a	66.7^{c} c
Freeze-dried	85.1^{d} b	92.1^{b} a	90.6^{b} a	58.8^{d} c
Spray-dried	91.7^{b} b	95.3^{a} a	93.7^{a} a	77.8^{b} c

LSD denotes least significant difference, $p < 0.05$, $N = 3$. LSD for means within the same row is 2.1, means followed by different full case letters within a row are significantly different. LSD for means within columns is 2.2, means followed by different lower case superscripts within a column are significantly different

We propose that both freezing and freeze-drying induce the formation of insoluble aggregates regardless of extraction temperature. Freezing is not instantaneous. Initially, only part of the water is frozen, increasing the protein concentration in the unfrozen water [21]. High protein concentrations induce protein–protein interactions and, as a consequence, larger aggregates [13]. In addition, during the sublimation of freeze-drying, the sublimation front moves down into the product and the ''liberated'' water molecules have to pass through a layer of dried product. Some of these molecules are adsorbed and allow molecular mobility for further aggregation [21]. On the other hand, spray-drying is a much faster process that does not allow for such interactions to occur. Spray-drying causes some denaturation, as evidenced by our thermal behavior and surface hydrophobicity data, but speed and shear in this process prevent the proteins from forming large aggregates. Similar shear–temperature treatment models have been previously proposed for hydrothermal processing [22]. The degree of denaturation and extent of aggregation depend on both extraction temperature and preservation method.

Surface Hydrophobicity

Many of the molecular and functional properties of food proteins are related to the relative proportions of hydrophobic and hydrophilic amino acids, and their spatial distribution in the primary structure [23]. The extent of the hydrophobic regions exposed by a given protein significantly affects intermolecular interactions, such as the binding of small ligands or associations with other macromolecules (including protein–protein or protein–lipid interactions), which in turn affect surface-active functional properties [24].

The SPIs extracted at higher temperatures (80 and 60 °C) had significantly higher surface hydrophobicities than did SPIs extracted at lower temperatures (25 and 40 °C) (Table 4). There were also significant differences in hydrophobicity between freeze-dried and spray-dried SPIs. Higher hydrophobicities were achieved with spray-drying than with freeze-dried SPI.

These results were consistent with DSC results indicating that SPIs extracted at 60 and 80 °C were more extensively denatured. Higher proportions of hydrophobic regions are exposed in these products, probably due to unfolding. This mechanism can also explain the higher hydrophobicities of spray-dried SPIs, but it fails to explain why the freeze-dried SPIs

Table 4 Effects of extraction temperature and preservation method on surface hydrophobicity (dimensionless) of soy protein isolate at pH 7.0

Treatment	Extraction temperature (°C)			
	25	40	60	80
Fresh	$278^{a,b}$ c	$275^{b,c}$ c	388^{b} b	425^{b} a
Frozen/thawed	262^{b} c	294^{b} b	389^{b} a	413^{b} a
Freeze-dried	205^{c} c	255^{c} b	339^{c} a	346^{c} a
Spray-dried	290^{a} c	323^{a} b	458^{a} a	470^{a} a

LSD denotes least significant difference, $p < 0.05$, $N = 3$. LSD for means within the same row is 29.8, means followed by different full case letters within a row indicate that means are significantly different. LSD for means within the same column is 23.7, means followed by different lower case superscript letters within a column are significantly different

had significantly lower hydrophobicities. One probable explanation is that the lower hydrophobicities may be due to aggregation, which would in turn prevent ANS from interacting with those aggregated hydrophobic sites. Electrostatic charges produced during spray-drying may partially account for the increased hydrophobicity measured by ANS [24].

There was interaction between extraction temperature and drying method for surface hydrophobicity, which had an *F*-value of 3.55, *p*-value of 0.0063, and 24 degrees of freedom for error. The interaction for hydrophobicity also fits our proposed model of higher extents of insoluble aggregates being formed by freezing and freeze-drying. Surface hydrophobicity not only depends on the extent of denaturation, which increases the surface hydrophobicity, but also on the extent of aggregation, which tends to decrease surface hydrophobicity [17]. The decrease in surface hydrophobicity due to freezing and freeze-drying can be explained by the formation of larger aggregates, which stops the ANS probe from reaching the hydrophobic regions exposed by denaturation. In contrast, the spray-dried samples, which have approximately the same degree of denaturation and higher solubility due to smaller aggregates, will have higher surface hydrophobicity.

Emulsification Capacity

Proteins are often used to aid emulsion formation and to increase the emulsion stability of foods. Proteins are much larger and more complex than simple emulsifier molecules. The formation of protein-stabilized emulsions requires that the protein molecule migrate to the water/lipid interface and unfold such that hydrophobic regions can contact the lipid phase [25]. In order to achieve this, protein molecules must have both hydrophilic and hydrophobic regions and retain flexibility in order to unfold.

SPIs extracted at 25 and 40 °C had the highest emulsification capacities, followed by SPIs extracted at 60 and 80 °C (Table 5). This indicates that emulsification capacity was influenced by the amount of native β-conglycinin present in the SPI and the protein solubility. Higher contents of native β-conglycinin and higher protein solubility favored higher emulsification capacities.

The emulsification capacities for freeze-dried and spray-dried SPIs were not significantly different from each other for any extraction temperature. On the other hand, there were significant differences between the emulsification capacities for fresh and freeze/thawed SPIs extracted at 25 °C.

There was no significant interaction between extraction temperature and drying method for emulsification capacity, with an *F*-value of 0.33, *p*-value of

Table 5 Effects of extraction temperature and preservation method on the emulsification properties of soy protein isolate at pH 7.0

Treatment	Extraction temperature (°C)			
	25	40	60	80
Emulsification capacity (g of oil emulsified by 1 g of SPI)[1]				
Fresh	546^{b} a	578^{a} a	$481^{a,b}$ b	455^{a} b
Frozen/thawed	587^{a} a	611^{a} a	517^{a} b	483^{a} b
Freeze-dried	$563^{a,b}$ a	585^{a} a	478^{b} b	459^{a} b
Spray-dried	590^{a} a	592^{a} a	$498^{a,b}$ b	475^{a} b
Emulsification activity (absorbance at 500 nm)[2]				
Fresh	0.248^{a} d	0.257^{a} c	0.285^{a} b	0.334^{a} a
Frozen/thawed	0.236^{b} d	0.252^{a} c	0.271^{b} b	0.322^{b} a
Freeze-dried	0.234^{b} d	0.252^{a} c	0.270^{b} b	0.311^{c} a
Spray-dried	0.233^{b} d	0.242^{b} c	0.264^{b} b	0.294^{d} a
Emulsification stability index (dimensionless)[3]				
Fresh	103^{c} b	117^{c} b	320^{a} a	335^{a} a
Frozen/thawed	155^{b} c	199^{a} b	170^{c} c	233^{c} a
Freeze-dried	112^{c} d	159^{b} b	142^{d} c	188^{d} a
Spray-dried	169^{a} d	190^{a} c	229^{b} b	253^{b} a

LSD denotes least significant difference, $p < 0.05$, $N = 3$

[1] LSD for means within the same row is 35.7, means followed by different full case letters within a row are significantly different. LSD for means within the same column is 36.1, means followed by different superscripts within a column are significantly different

[2] LSD for means within the same row is 0.009, means followed by different full case letters within a row are significantly different. LSD for means within the same column is 0.008, means followed by different superscripts within a column are significantly different

[3] LSD for means within the same row is 16.3, means followed by different full case letters within a row are significantly different. LSD for means within the same column is 15.7, means followed by different superscripts within a column are significantly different

0.9551, and 24 degrees of freedom. This indicated that the emulsification capacities for these SPIs were not affected by preservation method. When assessing emulsification capacity, any preservation method can be used. This was the only functional property that showed no significant interaction, indicating that both extraction temperature and preservation method had additive effects and were independent of each other.

Emulsification Activity and Emulsification Stability Index

Emulsions are thermodynamically unstable. Once formed, an emulsion can undergo a number of changes. It is of interest to know not only how efficient a protein dispersion is at emulsifying oil, but also the stability of the resulting emulsion. The factors involved in emulsification stability are many and complex [25].

The emulsification activities of SPIs extracted at 80 °C were significantly higher than for SPIs extracted at 60 °C, followed by SPIs extracted at 40 °C, and then by SPIs extracted at 25 °C (Table 5). The freeze-dried SPIs had higher emulsification activities than spray-dried SPIs, but this phenomenon was only significant for SPIs extracted at 40 and 80 °C. Fresh samples had the highest emulsification activities. There was significant interaction between the extraction temperature and preservation method, with an F-value of 3.77, p-value of 0.0044, and 24 degrees of freedom for error.

Emulsification stability indices were significantly affected by both extraction temperature and drying method. The SPIs extracted at higher temperatures had significantly better stabilities than those dried at lower temperatures (Table 5). Freeze-dried SPI had consistently lower emulsion stability indices compared to spray-dried SPI, but only SPIs extracted at 40 and 60 °C were significantly different. Preservation method affected emulsion stability index to different extents. This behavior corresponded to both solubility and surface hydrophobicity. In order to produce stable emulsions, the molecule must be soluble in the continuous phase and have sufficient hydrophobic patches exposed to the dispersed oil phase. There was significant interaction between extraction temperature and preservation method, with an F-value of 99.58, a p-value of <0.0001, and 24 degrees of freedom for error.

Foaming Properties

Extraction temperature and preservation method significantly affected foaming capacity. For fresh and frozen samples, the SPIs extracted at higher temperatures had higher foaming capacities (Table 6). For freeze-dried and spray-dried samples, SPIs extracted at 40 °C had the highest foaming capacities. Fresh or frozen products do not predict dry SPI behavior. There was significant interaction between extraction temperature and preservation method, with an F-value of 33.25, a p-value of <0.0001, and 24 degrees of freedom for error.

High K values indicate that a less stable foam is formed. Foaming stability was significantly affected by both extraction temperature and preservation method (Table 6). The foams prepared with SPI extracted at 80 °C were highly stable. This may be due to the higher surface hydrophobicities observed in these samples, which allow proteins to move more efficiently to the water/air interface and form more stable foams. Freeze-dried products and spray-dried SPIs were not significantly different from each other, except for rate of foaming. Similar foaming capacities and K values for spray-dried and freeze-dried samples suggest that surface hydrophobicity may not be the only factor involved. Native proteins (molecular flexibility) and solubility (mobility) may also be involved, since the protein must efficiently move to the water/air interface and be able to remain there to achieve stability. There was significant interaction between extraction temperature and preservation method, with an F-value of 132.27, a p-value of <0.0001, and 24 degrees of freedom for error.

Both extraction temperature and preservation method significantly affected the rate of foaming. The SPIs extracted at 80 °C formed foams fastest (Table 6). The freeze-dried SPI had significantly higher foaming rates than spray-dried SPI. Drying increased the rate of foaming in all cases. There was significant interaction between extraction temperature and preservation method, with an F-value of 4.05, a p-value of 0.0029, and 24 degrees of freedom for error.

Dynamic Viscosity

The SPIs extracted at 60 °C had the lowest consistency factors (k) and the closest flow behavior indexes (n) to Newtonian fluid behavior of all SPIs tested. This may be due to the fact that viscosity was controlled by the native β-conglycinin component, and when this protein was denatured, viscosity dropped. Another viscosity change was observed with thermal denaturation of the glycinin, which would account for the high viscosity obtained by the SPI extracted at 80 °C. Apparent viscosities for these products were similar to results reported by Rickert et al. [10] and were consistent with their findings for β-conglycinin, glycinin, and SPI. Upon β-conglycinin denaturation under alkaline

Table 6 Effects of extraction temperature and preservation method on foaming properties of soy protein isolate at pH 7.0

Treatment	Extraction temperature (°C)			
	25	40	60	80
Foaming capacity (mL of foam formed by ml of a 0.5 % SPI dispersion)[1]				
Fresh	1.218^{a} b	1.295^{a} b	1.437^{a} a	1.449^{a} a
Frozen/thawed	0.888^{b} b	1.114^{b} b	$1.319^{a,b}$ a	$1.387^{a,b}$ a
Freeze-dried	1.250^{a} b	1.377^{a} a	1.163^{c} b	1.192^{c} b
Spray-dried	1.250^{a} a,b	1.375^{a} a	$1.234^{b,c}$ b	$1.266^{b,c}$ a,b
Foaming stability [K = 1/(ml min)][2]				
Fresh	0.013^{a} a	0.013^{a} a	0.013^{a} a	0.008^{a} b
Frozen/thawed	0.008^{b} b,c	0.009^{b} a,b	$0.011^{a,b}$ a	$0.006^{a,b}$ c
Freeze-dried	0.005^{c} b	0.006^{c} b	0.009^{b} a	$0.004^{a,b}$ b
Spray-dried	$0.007^{b,c}$ b	$0.007^{b,c}$ b	$0.011^{a,b}$ a	0.007^{a} b
Rate of foaming (ml/min)[3]				
Fresh	15.34^{b} b	16.63^{b} b	16.80^{a} b	21.97^{b} a
Frozen/thawed	12.86^{c} b	13.26^{c} b	12.87^{b} b	19.43^{c} a
Freeze-dried	21.54^{a} b	19.15^{a} c	18.36^{a} c	28.41^{a} a
Spray-dried	16.31^{b} b	12.47^{c} c	13.36^{b} c	23.34^{b} a

LSD denotes least significant difference, $p < 0.05$, $N = 3$

[1] LSD for means within the same row is 0.127, means followed by different full case letters within a row are significantly different. LSD for means within the same column is 0.136, means followed by different superscripts within a column are significantly different

[2] LSD for means within the same row is 0.0024, means followed by different full case letters within a row are significantly different. LSD for means within the same column is 0.0020, means followed by different superscripts within a column are significantly different

[3] LSD for means with the same row is 2.35, means followed by different full case letters within a row are significantly different. LSD for means within the same column is 2.06, means followed by different superscripts within a column are significantly different

Table 7 Effects of extraction temperature and preservation method on dynamic viscosity soy protein isolate at pH 7.0

Treatment	Extraction temperature (°C)			
	25	40	60	80
Flow consistency Index (k, mPa s)[1]				
Fresh	0.27^{a} c	$1.25^{b,c}$ b	0.05^{a} c	9.11^{a} a
Frozen/thawed	0.38^{a} c	$2.05^{a,b}$ b	0.04^{a} c	7.56^{b} a
Freeze-dried	0.45^{a} c	2.32^{a} b	0.08^{a} c	6.27^{c} a
Spray-dried	0.65^{a} b	0.84^{c} b	0.03^{a} b	2.24^{d} a
Flow behavior index (n, dimensionless)[2]				
Fresh	0.675^{a} b	0.450^{b} c	0.925^{a} a	0.172^{d} d
Frozen/thawed	0.585^{b} b	0.389^{c} c	0.871^{c} a	0.369^{c} d
Freeze-dried	0.562^{c} b	0.380^{c} c	0.877^{c} a	0.392^{b} c
Spray-dried	0.513^{d} b	0.489^{a} c	0.897^{b} a	0.464^{a} d

LSD denotes least significant difference, $p < 0.05$, $N = 3$

[1] LSD for means within the same row is 0.90, means followed by different full case letters within a row are significantly different. LSD for means within the same column is 0.81, means followed by different superscripts within a column are significantly different

[2] LSD for means within the same row is 0.018, means followed by different full case letters within a row are significantly different. LSD for means within the same column is 0.019, means followed by different superscripts within a column are significantly different

conditions, β-conglycinin trimers dissociate into individual subunits [16], which causes the drop in viscosity. On the other hand, when glycinin is denatured under alkaline conditions it dissociates into acidic and basic polypeptide components, which in the presence of β-conglycinin will first form soluble aggregates of a heterogeneous nature [20]. Upon cooling they form highly organized complexes. Depending upon the protein concentration, they gel [26], which accounts for the high viscosity obtained with the 80 °C extraction treatment.

Dynamic viscosity was affected by both extraction temperature and preservation method to different extents (Table 7). Drying method significantly affected k for those SPIs that had higher viscosities (40 and 80 °C). For these samples, spray-drying produced the lowest consistency factors, which was consistent with solubility. In general, those samples with higher solubilities for the same extraction temperature were less viscous. For the SPIs prepared at 25 and 60 °C, there were no differences in consistency factor among preservation methods. Flow behavior index (n) gives an idea of how close to a Newtonian fluid the dispersions are; the closer to 1, the closer to a true Newtonian fluid behavior. In general, those SPI dispersions with high consistency factors had low flow behavior indexes.

There was significant interaction between extraction temperature and preservation method for dynamic viscosity, with an F-value of 2.32, and a p-value of 0.0479 for consistency factor (k), and an p-value of 175.16, and a p-value of <0.0001 for flow behavior index (n) with 24 degrees of freedom for error in both cases. Our proposed model also fitted viscosity data and it explains why there was significant interaction between extraction temperature and preservation method. The previous thermal history and the size and distribution of the soluble/insoluble aggregates in addition to the above-described complex association–dissociation behaviors of glycinin and β-conglycinin account for this interaction.

Integration of Temperature and Preservation Data

The functionality of SPI was significantly affected by both the temperature at which the soybean flour was extracted and the method used for preservation. As extraction temperature increased, solubilities and

emulsification capacities decreased; surface hydrophobicities, emulsification activities and stabilities, and dynamic viscosities increased; and foaming properties improved. Denaturation enthalpies of the spray-dried and freeze-dried SPIs were similar. Spray-dried SPIs had higher solubilities, surface hydrophobicities, and emulsification stabilities and lower viscosities, emulsification activities and rates of foaming than did freeze-dried SPIs. Emulsification and foaming capacities and foaming stabilities were the same for both methods of drying. There was significant interaction between extraction temperature and preservation method for all functional properties tested except for emulsification capacity. We believe that the size and extent of aggregation account for the interaction between preservation method and extraction temperature.

Acknowledgments This journal paper of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA, USA, Project No. 6571, was supported in part by USDA National Research Intiative grant no. 2001-35503-10814, the Center for Crops Utilization Research, and by Hatch Act and State of Iowa funds.

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