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ORIGINAL PAPER

Functional Properties of Soy Protein Isolates Produced from Ultrasonicated Defatted Soy Flakes

Bishnu Karki · Buddhi P. Lamsal · David Grewell · Anthony L. Pometto III · J. van Leeuwen · Samir K. Khanal · Stephanie Jung

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Abstract This study aimed to determine the effect of pretreating defatted soy flakes with ultrasound on soy protein isolate (SPI) yield and functionality. Defatted soy flakes dispersed into water (16%, w/w) were sonicated for 30, 60 and 120 s at ultrasonic amplitudes of 21 and 84 μm_{pp} (peak to peak amplitude in μm), representing low and high power, respectively. The power densities were 0.30 and 2.56 W mL^{-1}, respectively. The SPI yield increased by 13 and 34%, after sonication for 120 s at low and high power, respectively. The sonication of defatted soy flakes for 120 s at the higher power level improved the SPI solubility by 34% at pH 7.0, while decreasing emulsification and foaming capacities by 12 and 26%, respectively, when compared to control SPI. Rheological behavior of the SPI was also modified with significant loss in consistency coefficient due to sonication. Some of these results could be explained by the loss of the protein native state with increased sonication time and power.

Keywords Defatted soy flakes · Ultrasound · Functional properties · Soy protein isolate

B. Karki · J. van Leeuwen
Environmental Science Program,
Iowa State University, Ames, IA 50011, USA

B. P. Lamsal · J. van Leeuwen · S. Jung (✉)
Department of Food Science and Human Nutrition,
Iowa State University, 1436 Food Sciences Building,
Ames, IA 50011, USA
e-mail: jung@iastate.edu

D. Grewell · J. van Leeuwen
Department of Agricultural and Biosystems Engineering,
Iowa State University, Ames, IA 50011, USA

D. Grewell · J. van Leeuwen
Department of Civil,
Construction and Environmental Engineering,
Iowa State University, Ames, IA 50011, USA

A. L. Pometto III
Department of Food Science and Human Nutrition,
Clemson University, Clemson, SC 29634-0316, USA

S. K. Khanal
Department of Molecular Biosciences and Bioengineering,
University of Hawaii at Mānoa, Honolulu, HI 96822, USA

Introduction

Soy protein isolate (SPI) is a commercial soy protein product having at least 90% protein [dry basis (db)] [1]. SPI holds a unique place in the human diet, not only because of its use as a low-cost substitute for animal food proteins, but also because of the health benefits associated with soy protein consumption, including blood cholesterol reduction and cardiovascular disease prevention [2]. SPI can also be used in non-food applications including the production of biodegradable plastics and paper coatings and sizing [3]. The versatile uses of SPI can be attributed to the wide range of functional properties that SPI can confer to a food product [4, 5]. These properties are affected by the intrinsic, extrinsic and process parameters [6], and could be modified by using alternative processing techniques [5]. Extensive research has been done on enzymatic [7–9], mechanical [5] and thermal [1, 10] modifications of soy proteins to improve their functional properties.

The first step in SPI production is an alkaline aqueous extraction of the defatted soy flakes. This extraction step is far from being efficient as about half of the available protein remains in the insoluble fraction [9]. Microwave heating [11], enzymatic [9] and chemical modifications were applied

to the defatted soy flakes, improving the protein extraction yield by up to 50% during the aqueous alkali extraction. High power ultrasound has recently been reported as a powerful method to increase extraction of intracellular compounds from plant materials, including oil from soy flakes [12], water-soluble polysaccharides from roots of valerian (*Valeriana officinalis* L.) [13] and saponin from ginseng [14]. While high power ultrasound seems to be a promising technology in improving the extraction efficiency of plant material, there is little information as to whether ultrasound-assisted extraction alters the physicochemical and biological properties of plant extracts, especially proteins. During ultrasonication, a mechanical effect occurs; therefore protein might undergo structural changes [15] that could result in changes in the SPI functional properties.

The objectives of this study were firstly, to determine the effects of sonication power and time applied on the SPI yield. Secondly, the functional properties and some structural properties of the SPI obtained from sonicated defatted soy flakes were determined.

Experimental Procedures

Defatted Soy Flakes

Hexane-defatted soy flakes were obtained from the Center for Crops Utilization Research (CCUR), Iowa State University (Ames, IA, USA). The soy flakes were stored in air-tight plastic bags at 4 °C until use. The moisture content of the soy flakes was 5.2%.

Ultrasound Treatment

Defatted soy flakes (100 g) were dispersed into 500 mL tap water in a customized 1.2-L stainless steel sonication chamber. The samples were treated in batch-mode using a Branson 2000 Series bench-scale ultrasonic unit (Branson Ultrasonics Corporation, Danbury, CT, USA), with a maximum power output of 2.2 kW. A standard 20-kHz half wavelength titanium horn with a gain of 1:2.8 and a booster with a gain of 1:1.5 were used. Samples were sonicated at two different amplitude (power) levels, 21 and 84 μm_{pp} (peak to peak amplitude in μm), and designated as low and high power level, respectively. The power levels were changed by varying the amplitude at the horn tip through pulse-width modulation voltage regulation to the converter. For each power input (amplitude), the samples were sonicated for 30, 60 and 120 s without temperature control. The temperature of the slurry was 25, 26 and 28 °C, after sonication at a low power level for 30, 60 and 120 s, respectively. At a high power level, the temperature of slurry was 31, 48 and 65 °C after sonication for 30, 60 and 120 s, respectively.

Extraction and SPI Procedures

After sonication, 500 mL of water at 65 °C was added to the flakes slurry to obtain a flakes-to-water ratio of 1:10 (w/w). Controls were similarly prepared from unsonicated soy flakes (Fig. 1). The initial pH of approximately 6.2 was raised to 8.5 by adding 2N NaOH. The slurry was placed into a 60 °C water bath and stirred for 30 min while maintaining a constant pH. The sample was then centrifuged at 10,000 × *g* for 20 min at 20 °C. The supernatant was acidified to pH 4.5 with 2N HCl and stored at 4 °C for 1 h, before centrifuging at 10,000 × *g* for 20 min at 20 °C. The supernatant was discarded while the curd (precipitate) was collected. The precipitate was washed two times with distilled water and centrifuged again at the same condition. The washed precipitate was dispersed into distilled water and neutralized to pH 7.0 with 2N NaOH. The neutralized curd was freeze-dried, powdered, sealed in plastic bag and stored at 4 °C until use. These procedures were performed in triplicate.

Protein and Moisture Determination

The protein contents of the SPI and soy flakes were determined with a Nitrogen Analyzer (Rapids N III, Elementar Americas, Inc., Mt. Laurel, NJ, USA) following the Dumas method [16] with aspartic acid (A9, 310-0; Sigma–Aldrich, St. Louis, MO, USA) as the nitrogen reference calibration. The crude protein content was calculated from the nitrogen content of the material using a nitrogen conversion factor of 6.25.

The protein yield was calculated as:

$$\text{Protein yield } (\%) = \left[\frac{\text{weight of protein in isolates (g)}}{\text{weight of protein in defatted flakes (g)}}\right] \times 100$$

For moisture determination, approximately 1.0 g of sample was heated in a vacuum-oven at 110 °C for 3 h and the moisture content then determined gravimetrically [17].

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was performed on isolated soy proteins obtained from sonicated and non-sonicated defatted soy flakes using an SDS-Tris-glycine buffer system with Bio-Rad Ready Gels (Mini Protean II Gel, Bio-Rad Inc., CA). Protein samples were diluted to a concentration of 1 mg mL^{-1} in a solution containing 125 mM THAM, 5.0 M urea, 2% β-mercaptoethanol, 0.20% SDS, 20% glycerol, 0.01% bromophenol blue and pH 6.8. Ten micrograms of an MW marker (Sigma M4038) and 5 μg of protein sample were loaded into a lane. Gel electrophoresis was carried out at a constant 200 V. The gels were stained according to the method of Neuhoff et al. [18].

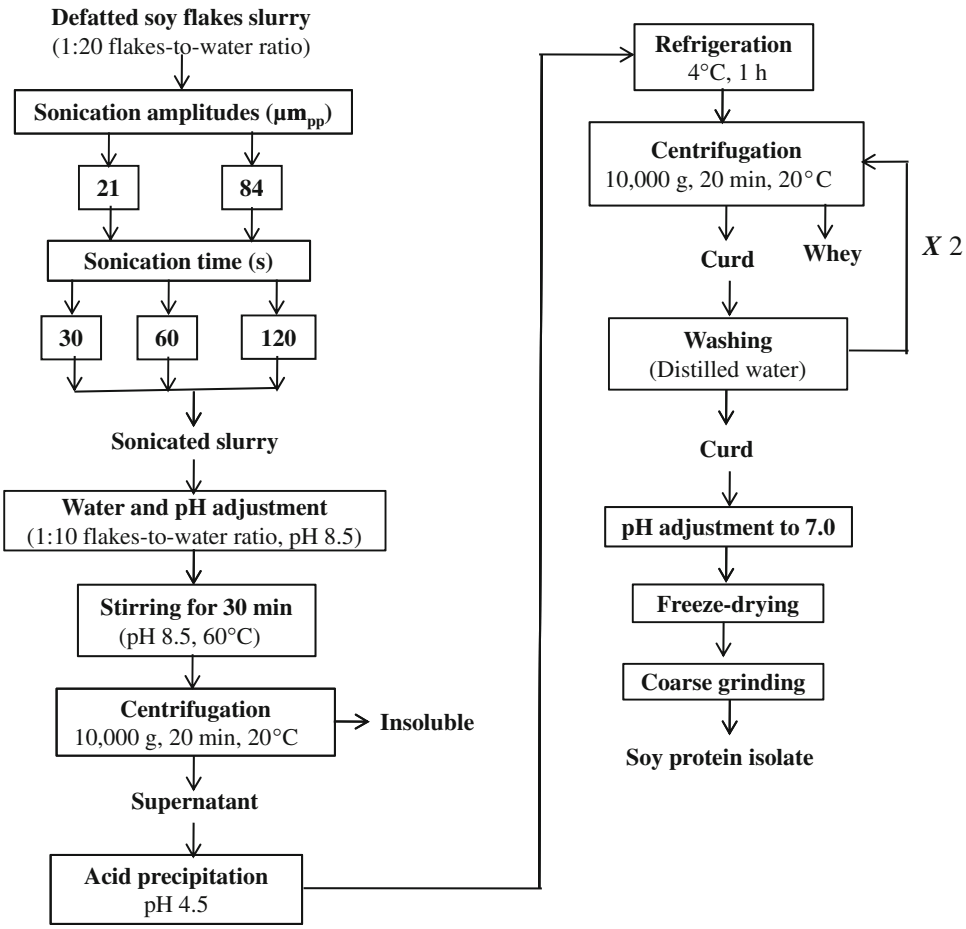

Fig. 1 Flow chart of sonication conditions and soy protein isolate production

Thermal Behavior

Differential scanning calorimetry (DSC) measurement of the isolated protein was performed with a DSC 7 Perkin-Elmer Thermal Analyzer (PerkinElmer, Inc., Shelton, CT, USA). Twenty-five microliters of 10% (w/w, db) protein dispersion prepared in 0.01 M phosphate buffer (pH 7.0) was hermetically sealed in an aluminum pan and an empty sealed pan was used as reference. The sample was heated from 10 to 120 °C at a rate of 10 °C min^{-1}. Peak denaturation temperature (T_d in °C) and thermal denaturation enthalpies (ΔH in Joules per gram of protein) were calculated from the endothermic curves using Pyris software (version 7.0, PerkinElmer, Inc., Shelton, CT, USA). T_d is the maximum temperature of the peak in the curve and ΔH is the area under the endothermic curve. All samples were analyzed in triplicate.

Water Solubility Profile

An aliquot of 0.50 g of freeze-dried protein was dispersed into distilled water at a 1% concentration. The pH of the protein dispersion was adjusted to 3, 4, 5, 7 and 9 with either 2N HCl or 2N NaOH. The protein dispersion was stirred for 1 h and within this hour the pH was adjusted, if necessary, after 15 and 30 min of stirring. Twenty-five milliliters of the dispersion was loaded into 50-mL centrifuge tubes and centrifuged at 10,000×g for 10 min at 20 °C. The supernatant was measured for protein content using the Biuret method with bovine serum albumin (BSA) as standard. Solubility was calculated as:

$$\text{Protein solubility } (\%) = \left[\frac{\text{Protein in supernatant (g)}}{\text{Protein in starting material (g)}}\right] \times 100$$

All samples were analyzed in triplicate.

Emulsification Capacity (EC)

Twenty-five milliliters of a 2% (w/w) sample dispersion adjusted to pH 7.0 with 2N HCl or 2N NaOH was transferred to a 400-mL plastic beaker. Canola oil dyed with approximately 4 μg mL^{-1} Sudan Red 7B (Sigma, St. Louis, MO, USA) was continuously blended into the

dispersion at a 36 mL min^{-1} flow rate by using a Bamix hand mixer (ESGE AG Model 120, Mettlen, Switzerland) at low setting until phase inversion was reached. Phase inversion was identified by the abrupt decrease in homogeneity and loss of viscosity. The weight of oil needed to reach the phase inversion was determined and the EC (g oil/g sample) was calculated as the weight of oil used to cause inversion multiplied by 2. All samples were analyzed in triplicate.

Foaming Capacity (FC)

A dispersion of 0.5% (w/w, db) of soy protein at pH 7.0 was prepared. An 80-mL aliquot was loaded into a custom-designed glass column with a coarse fritted glass disk at the bottom and N_2 gas was purged through the sample at a 100 mL min^{-1} flow rate. The time for the foam to reach a 300-mL volume, the volume of the liquid incorporated into the foam, and the time for one half of the liquid incorporated into the foam to drain back were measured. The following parameters were calculated:

$$\text{Foaming capacity} = \left[\frac{V_f}{f_r \times t_f}\right]$$

$$\text{Foaming stability} = \left[\frac{1}{(V_{max} \times t_{1/2})}\right]$$

$$\text{Foaming rate} = \left[\frac{V_{max}}{t_f}\right]$$

where V_f is a fixed volume of 300 mL, f_r is the flow rate of the gas and t_f is time to reach V_f, V_{max} is volume of liquid incorporated into foam and $t_{1/2}$ is the time to drain one half of the liquid incorporated into the foam. All the samples were analyzed in triplicate.

Rheological Properties

A 10% protein dispersion of SPI was prepared in distilled water at pH 7.0. The sample was analyzed with a RS-150 Rheo Stress Rheometer (Haake, Germany) equipped with a cone-plate sensor of 60 mm diameter and 2° angle. The shear was applied at a rate of 10–500 s^{-1} at room temperature. The power law model, $\sigma = k\left(\dot{\gamma}\right)^n$ was used to model the experiment flow curves, where σ was the shear stress (Pa), k was the consistency coefficient (mPa.s^n), $\dot{\gamma}$ was the shear rate (s^{-1}) and n was the flow behavior index. All samples were measured in triplicate.

Statistical Analysis

Three independent batches of SPIs were prepared from defatted soy flakes for each sonication condition (power level and sonication time) and used for experiments. Data were analyzed by using the General Linear Model (GLM) in SAS system (version 9.1, SAS Institute, Inc., USA) to compare means and calculate least significant difference (LSD) at $p < 0.05$.

Results and Discussion

Protein Extraction Yield and Protein Content of SPI

The protein yield significantly increased with increase in sonication time and power level as compared to the control (Table 1). Protein extraction yield increased by 13 and 34% when defatted soy flakes were treated for 120 s at low and high sonication power level, respectively. This increase could be attributed to cavitation induced by the ultrasound treatment, promoting a turbulent flow and enhancing mass transfer of the cell content [19]. The physical damage caused by the hydrodynamic shear force produced by the cavitational effect of the ultrasound might also have contributed to the protein extractability increase [20]. The best condition for protein extractability, i.e., high power for 120 s was, however, not favorable to a high protein content of the recovered isolated proteins. Indeed this SPI had a significantly lower protein content than the one obtained for SPI from untreated flakes, 85% versus 89%. While the appellation SPI could only be used if protein content is higher than 90%, db [21], this term was used for isolated proteins from high power sonication applied for 60 and 120 s, to simplify the description of the isolated protein fractions. The protein content of SPI being affected by the amount of associated and conjugated non-protein constituents precipitating as impurities with the protein [22], the lower protein content of SPI at high power and longer sonication time could be explained by an

Table 1 Protein yield and protein content of soy protein isolates as a function of sonication conditions

Sample	Sonication time (s)	Protein yield (%, db)	Protein content (%, db)
Control	0	54.24 ± 0.40^{a}	89 ± 0.80^{a}
Low power level	30	57.27 ± 1.23^{b}	$90 \pm 1.52^{a,b}$
	60	58.23 ± 0.80^{b}	94 ± 0.30^{c}
	120	61.28 ± 1.05^{c}	91 ± 1.48^{b}
High power level	30	62.39 ± 0.98^{c}	91 ± 0.82^{b}
	60	65.89 ± 1.53^{d}	86 ± 0.41^{d}
	120	72.91 ± 0.65^{e}	85 ± 1.30^{d}
LSD		1.42	1.45

LSD least significant difference; *db* dry basis

Means within each column followed by *different superscript* are significantly different at $p < 0.05$ ($n = 3$)

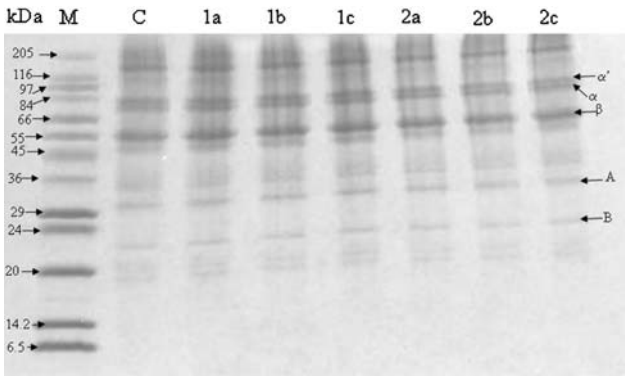

Fig. 2 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profiles of soy protein isolates. *M* molecular weight marker; *C* control; *1a*, *1b* and *1c* SPI sonicated at low power level for 30, 60 and 120 s, respectively; *2a*, *2b* and *2c* SPI sonicated at high power level for 30, 60 and 120 s, respectively; α', α and β subunits of β-conglycinin; *A* acidic subunit of glycinin; *B* basic subunit of glycinin

increase in extracted non-protein constituents. The ultrasound-treated proteins solubility might also have increased at pH 4.5, i.e., pH applied for SPI production. A non-linear increase in protein extractability was observed with sonication power and sonication time. For instance, at 25% power output, i.e., low power level, as sonication time increased from 60 to 120 s, the mean protein extraction yield increased by 3% (from 58 to 61%) instead of 7% at 100% power output, i.e., high power level (from 66 to 73%). The nonlinearity of the effects of sonication power and sonication time on wheat flour protein extractability was previously reported by Singh et al. [23].

SDS-PAGE

SDS-PAGE profiles of isolated soy proteins obtained with sonication and without sonication of defatted soy flakes are shown in Fig. 2. The profile of the SPI recovered from treated defatted soy flakes was similar to the one of the control, revealing that sonication did not modify the peptide profile, regardless of the sonication conditions.

Thermal Properties of SPI

The effect of sonication conditions (power and time) on the thermal properties of SPI is shown in Table 2. The control SPI exhibited two thermal transitions at approximately 77 and 92 °C corresponding to the denaturation peak temperature (T_d) of β-conglycinin (7*S*) and glycinin (11*S*), respectively [24, 25]. The enthalpy (ΔH) value of β-conglycinin and glycinin for control SPIs, 0.98 and 5.19 J g^{-1}, respectively, were consistent with those of Deak and Johnson obtained at the same extraction temperature of 60 °C [4]. After 60 s at low power level, T_d of β-conglycinin and glycinin decreased significantly, while increasing after 120 s at high power level, when compared to the control, suggesting protein conformation changes [25]. At low power level, the ΔH of β-conglycinin and glycinin remained unchanged, except after 60 s for β-conglycinin, where an unexpected increase was observed. Treating the β-conglycinin at a high power level further decreased its ΔH value. Similarly, ΔH of glycinin decreased with treatment at high power level, the treatment applied for 120 s decreasing the value to 1.73 compared to 5.19 J g^{-1} for the control. This decrease could not be attributed to the increase of the temperature of the extraction medium during treatment (up to ~65 °C), as T_d of the protein in this condition was 95.11 °C, and suggested some conformational change in the protein as previously observed for ultrasonicated bovine serum albumin [15]. Our results therefore suggested that increased extractability and changes in protein conformation might occur simultaneously, one of this phenomenons being more important than the other depending on the processing conditions. These results are supported by previous studies

Table 2 Thermal properties of soy protein isolates as a function of sonication conditions

Sample	Sonication time (s)	Peak temperature, T_d (°C)		Enthalpy, ΔH (J g^{-1})	
		β-Conglycinin	Glycinin	β-Conglycinin	Glycinin
Control	0	76.66 ± 0.43^{a}	92.32 ± 0.60^{a}	$0.98 \pm 0.06^{a,d}$	5.19 ± 0.30^{a}
Low power level	30	75.60 ± 0.52^{b}	92.72 ± 1.00^{a}	$0.80 \pm 0.26^{a,d}$	4.95 ± 0.78^{a}
	60	73.16 ± 0.47^{c}	90.08 ± 0.83^{b}	1.37 ± 0.30^{b}	$4.73 \pm 0.48^{a,b}$
	120	$77.15 \pm 0.90^{a,d}$	91.80 ± 0.50^{a}	$0.81 \pm 0.12^{a,d}$	5.22 ± 0.12^{a}
High power level	30	77.33 ± 0.40^{d}	92.91 ± 1.24^{a}	0.39 ± 0.14^{c}	4.21 ± 0.61^{b}
	60	$76.81 \pm 0.71^{a,d}$	93.03 ± 0.49^{a}	0.71 ± 0.15^{d}	4.37 ± 0.51^{b}
	120	78.18 ± 0.31^{d}	95.11 ± 0.34^{c}	$0.83 \pm 0.24^{a,d}$	1.73 ± 0.23^{c}
LSD		0.472	0.740	0.196	0.578

LSD least significant difference

Means within each column followed by *different superscript* are significantly different at $p < 0.05$ ($n = 3$)

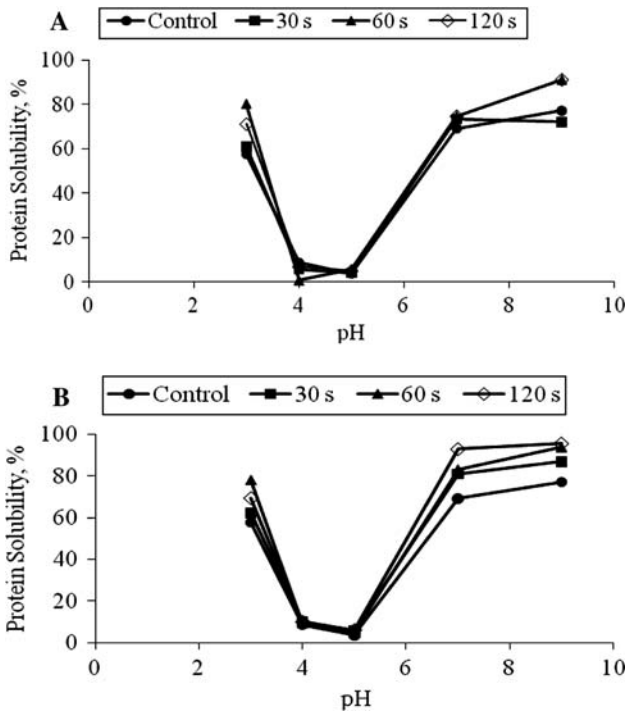

Fig. 3 Protein solubility of soy protein isolates in water as a function of pH at different sonication conditions. **a** Low power level (21 μm); **b** high power level (84 μm)

reporting increased extractability [12] and changes in protein structure after ultrasound [15]. They could be explained by the cavitational effect of the ultrasound, a phenomenon involving the formation and collapse of minute gas bubbles [20]. The amount of mechanical energy transferred into the liquid system largely depends on the experimental conditions and may usually give unpredictable results. Thus, our findings indicated that sonication of defatted soy flakes did not completely denature the proteins recovered in the SPI; however, denaturation occurred to some extent with an increase in sonication power and sonication time.

Protein Solubility

All samples exhibited the typical U-shaped solubility profile characteristic of soy proteins, with the lowest solubility at the isoelectric pH (4–5) (Fig. 3). Regardless of sonication conditions, increased solubility of the treated SPI was observed at pH 3.0, 7.0 and 9.0, while for other pH values and treatment conditions, no such increment pattern was observed. Among the tested samples, the SPI obtained from sonication of defatted soy flakes for 120 s at a high power level had the highest solubility (~95%) at pH 9.0. This increase in solubility could not be attributed to the changes in the peptide profiles as none of the treatment altered the SDS-PAGE profile of the samples (Fig. 2). Thus, part of the increased solubilities for the samples treated under the more drastic conditions can be related to the denaturated state of the proteins (Table 2), which might have contributed to changes in the number of hydrophobic residues, charge, electrostatic repulsion and ionic hydration, which are parameters affecting protein solubility [26]. In principle, it is usually understood that denatured protein has a lower solubility as compared to native protein; however, Zheng et al. [27] also reported the higher solubility of fully denatured protein as compared to partly denatured and native spray-dried proteins, suggesting that protein solubility is influenced by several factors not only by the degree of exposed hydrophobic groups.

Emulsification Capacity and Foaming Properties

The average emulsification capacity (EC) obtained for the control SPI was 445 g oil/g protein at pH 7.0. Under some conditions (low power for 60 and 120 s and high power for 120 s), the EC of the recovered SPIs was significantly decreased (Fig. 4), while for other conditions, the SPIs retained their EC. No clear correlation could be made with DSC results (Table 2) or protein solubility at pH 7.0 (Fig. 3), which increased regardless of the treatment.

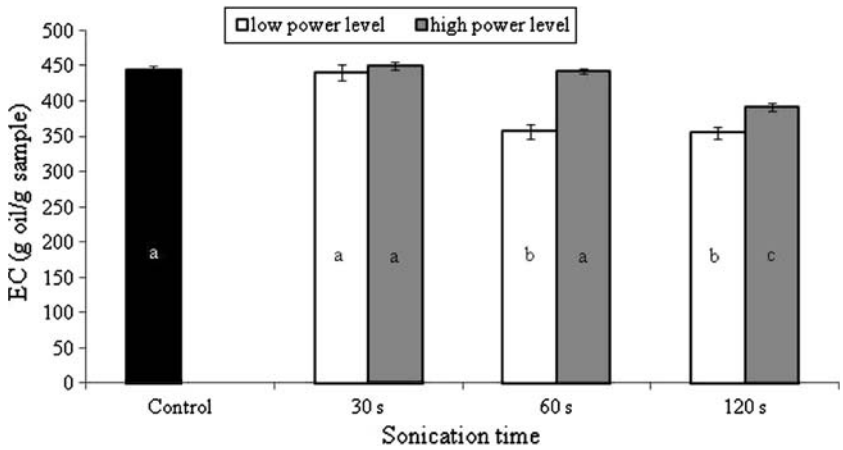

Fig. 4 Emulsification capacity of soy protein isolates under various sonication conditions. Emulsification capacities bearing the *same letters* are not significantly different at $p < 0.05$ ($n = 3$)

Table 3 Foaming properties of soy protein isolates under various sonication conditions

Sample	Sonication time	FC[A]	FR (mL min^{-1})	FS (1/mL min^{-1})
Control	0	0.98 ± 0.06^{a}	8.30 ± 0.51^{a}	0.0121
Low power level	30	0.83 ± 0.01^{b}	7.00 ± 0.18^{b}	0.0106
	60	0.81 ± 0.05^{b}	6.82 ± 0.26^{b}	0.0117
	120	0.76 ± 0.05^{c}	6.92 ± 0.63^{b}	0.0103
High power level	30	0.71 ± 0.05^{d}	6.11 ± 0.71^{c}	0.0100
	60	0.84 ± 0.01^{b}	6.66 ± 0.41^{b}	0.0119
	120	$0.72 \pm 0.02^{c,d}$	6.19 ± 0.49^{c}	0.0101
LSD		0.05	0.56	Not different

LSD least significant difference; FC, FR and FS represents foaming capacity, foaming rate and foaming stability of SPI

Means within each column followed by *different superscript* are significantly different at $p < 0.05$ ($n = 3$)

[A] Represents mL of foam formed by 1 mL of a 0.5% SPI dispersion

Table 4 Rheological properties of soy protein isolates for different sonication conditions

Sample	Sonication time (s)	Flow consistency index (k, mPa.s^{n})	Flow behavior index (n)	Apparent viscosity (Pa s) at 500 s^{-1}
Control	0	0.232 ± 0.020^{a}	0.626 ± 0.008^{a}	0.03 ± 0.001^{a}
Low power level	30	0.199 ± 0.020^{b}	0.641 ± 0.010^{a}	0.05 ± 0.001^{b}
	60	0.188 ± 0.004^{b}	0.663 ± 0.010^{b}	0.02 ± 0.001^{c}
	120	$0.175 \pm 0.005^{b,c}$	0.789 ± 0.002^{c}	0.05 ± 0.001^{b}
High power level	30	$0.170 \pm 0.001^{b,d}$	0.814 ± 0.010^{d}	0.02 ± 0.001^{c}
	60	0.148 ± 0.006^{c}	0.721 ± 0.010^{e}	0.03 ± 0.002^{a}
	120	$0.157 \pm 0.02^{c,d}$	0.665 ± 0.020^{b}	0.02 ± 0.001^{c}
LSD		0.02	0.01	0.001

LSD least significant difference

Means within each column followed by *different superscript* are significantly different at $p < 0.05$ ($n = 3$)

The foaming capacity (FC) of the control SPI was 0.98 ± 0.08 at pH 7.0. The FC and foaming rate (FR) of SPI were decreased after ultrasound treatment of the flakes at low and high power levels (Table 3). However, no change in foaming stability (FS) of SPI was observed after sonication treatment to defatted soy flakes. Although an increase in protein solubility would increase the EC and FC [28], sonication might have altered the conformation of the protein in a way that prevents the ability of protein to unfold at the interface resulting in poor surface activity.

Rheological Properties

The power law parameters (consistency coefficient k, and flow behavior index, n) were determined from the flow behavior of SPI prepared from non-sonicated and sonicated defatted soy flakes. All SPI dispersion exhibited a shear-thinning non-Newtonian flow behavior ($n < 1$) up to 500 s^{-1}. Ultrasound treatment of the soy flakes significantly decreased the SPI consistency and increased their flow behavior index except for the sample treated for 30 s at low power level. Some of these changes could be due to the effect of the treatment on the protein native state [4]. The changes in apparent viscosity of the SPIs at 500 s^{-1} shear rate are reported in Table 4. Some samples (60 s at low power and 30 and 120 s at high power level) had a lower apparent viscosity as compared to the control, which might be due to the increased protein solubility (Fig. 3) following sonication. Compared to the control, the apparent viscosity of SPI remained unchanged after sonication for 60 s at a high power level but the viscosity increased after 30 and 120 s sonication at low power level. Under these last conditions, proteins probably underwent some structural changes such as increased hydration properties [28] that impacted the viscosity behavior without modifying DSC values and peptide profile, as reported in the preceding sections.

Conclusions

The results of this study show that high power ultrasound is an efficient tool for improving the recovery of soy protein isolate from defatted soy flakes while only slightly modifying some functional properties including solubility and emulsification and foaming capacities. These changes were

made without peptide profile changes and with some alterations in the native state of glycinin and β-conglycinin as illustrated by the DSC results, which is a unique advantage of this technology compared to protease treatment of soy flakes, which increases protein extraction yield but also dramatically affects the functionality of the recovered proteins.

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