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Physical properties of ultrasound treated soy proteins

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ABSTRACT

The aim of this study was to examine the effect of ultrasound treatment on physical properties of soy proteins. For this purpose, soy protein isolates (SPI) and soy protein concentrate (SPC) were treated with ultrasound 20 kHz probe and ultrasound baths (40 and 500 kHz) system. In this study ultrasound treatment affected significant changes in texture of model systems prepared with soy protein concentrates, that gelled during ultrasound treatment with probe 20 and 40 kHz bath for 15 min. Model system prepared with SPI creamed during ultrasound treatment with probe 20 kHz for 15 min. Treatment with 20 kHz probe ultrasound lead to significant changes in conductivity, increased solubility for SPC, significantly increased specific surface area that is of interest in food texture and increased values of emulsion activity index. Weight mean diameter and volume–surface average diameter decreased significantly for all samples and all treatments. Flowing behaviour of SPI and SPC model systems has been greater influenced by ultrasound treatment. There was no improvement in foaming and emulsifying properties of soy protein model systems after 500 kHz bath treatment.

1. Introduction

Application of ultrasound in food industry is attracting much attention nowadays. The basic idea of application of ultrasound and sonochemistry in food processing lies in the fact that power ultrasound can cause changes in some properties (chemical, functional, physical etc.,) that may be of interest as technological benefit. Ultrasound represents mechanical waves, i.e. a variation of pressure or density with frequencies above the human hearing threshold (ca. 18 kHz) (Mason, 1998). Ultrasound can be classified in two categories: low-intensity (high frequency-low power), and high intensity (low frequency-high power) ultrasound. The low-intensity ultrasound uses very small power levels, typically less then 1 W cm^{-2}, with the frequency range of 5–10 MHz (McClements, 1995; Mason, 1998). It is generally used in diagnostic analysis of food materials. At high intensities (the high intensity ultrasound uses much higher power levels, typically in the range of 10–1000 W cm^{-2}, with the frequency of 20–100 kHz (Mason, 1998)), ultrasound has a lethal effect on microorganisms, and so has potential as a food preservation treatment (Entezari et al., 2004). High-intensity ultrasound is used in many food applications, such as emulsifying, sterilizing, extracting, degassing, filtrating, drying, and enhancing oxidation (Leadley and Williams, 2002; Mason, 1998). High intensity ultrasound generated by periodic mechanical motions of a probe, transfers ultrasonic energy into a fluid medium and triggers extremely high alterations in pressure leading to the formation of small rapidly growing bubbles (cavities) (Mason, 1990), which expand during the negative pressure excursion, and implode violently during the positive excursion generating high temperatures, pressures and shear forces at the probe tip (Suslick, 1988). This phenomenon is known as cavitation. During implosion, very high temperatures (approximately 5500 K) and pressures (approximately 50 MPa) are reached inside these bubbles (Mason, 1990; 1998; Suslick, 1988) that is consequently causing several reactions around imploding bubble.

Soy protein isolates (SPI) and soy protein concentrate (SPC) are used in many food products. The major components of soy proteins are storage proteins known as *β-conglycinin* and *glycinin*, which account for 65–80% of total seed proteins (Nielsen, 1997). According to their rate of sedimentation during centrifugation, soy proteins can be classified as 2S, 7S, 11S, and 15S. Form of isolate used in a specific food application varies according to its characteristics such as solubility, gelation, emulsification, dispersibility, viscosity (Orthoefer, 1978; Richert and Kolar, 1987; Soy Protein Council, 1987). Soy proteins are high in the amino acids glycine and arginine, which decrease cholesterol and lower insulin levels (Burrington, 2000). Soy proteins regulate appetite/satiety, weight control, enhance immune defences, prevent cavities, decrease chances of heart disease, decrease menopausal symptoms, increase mental alertness, develop and maintain healthy bones, decrease chances of developing cancer and also the protein samples are low in fat/fat-free, cholesterol free and lactose free (Russell, 2004).

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There are few papers that deal with whey proteins (Wang et al., 2008; Jambrak et al., 2008; Guzey et al., 2006), but none that deals with functional properties of soy proteins with ultrasound treatment used in food industry. Ultrasound was used very successfully in modifying solubility, foaming and other functional properties of whey proteins (Jambrak et al., 2008). Industrial implication could include ultrasound processing in the way of producing creams, pastes and other kind of product based on soy proteins. The fact that this procedure is less time and energy consuming ensure one of the possible usages of this technology in food industry. The aim of this study was to examine the effect of ultrasound treatment on physical properties of soy proteins. Namely, solubility, rheological, foaming and emulsifying properties, as well as specific surface area and specific diameters of particles have been measured with the aim to examine the influence of ultrasound treatment.

2. Materials and methods

2.1. Materials

Protein powders were purchased as declared by manufacturer. Protein powders were: *Soy protein isolates* (SPI, SUPRO® 595, Solae™) and *Soy protein concentrates* (SPC, ARCON® S, Solae™). According to the manufacturers, the typical composition of these powders was for soy protein concentrate: protein 66%, fat 3%, carbohydrate 21%, ash 4% and moisture 5%, and for soy protein isolate: protein 90%, fat 1%, carbohydrate 0%, ash 4%, and moisture 5%.

2.2. Sample preparation

The model systems marked as SPI and SPC were aqueous suspensions of powdered soy protein isolate and soy protein concentrate containing 10.0% (w/w) of dry matter. For this purpose appropriate amount of sample were dispersed in distilled water in volume of 100 ml by vigorous hand mixing until homogenous suspensions were obtained.

Soy protein model systems were marked as follows:

No ultrasound (A); 20 kHz probe – 15 min (B1); 20 kHz probe – 30 min (B2);

40 kHz bath – 15 min (**C1**); 40 kHz bath – 30 min (**C2**);

500 kHz bath – 15 min (**D1**); 500 kHz bath – 30 min (**D2**).

2.3. Ultrasound treatment

2.3.1. Ultrasound treatment with 20 kHz probe

Samples for ultrasound treatment with probe (20 kHz) were placed in 100 ml flat bottom conical flask. Samples were treated for 15 and 30 min with power ultrasound, high intensity and low frequency, 20 kHz probe (Model: V1A, power 600 W, Sonics & Materials Inc. Danbury CT, USA), attached to the transducer (Jencons Scientific Ltd. – Ultrasonic processor, Leighton Buzzard, United Kingdom) so that high power intensity can be obtained. Probe has a vibrating titanium tip 1.2 cm and is immersed in the liquid and the liquid is irradiated with an ultrasonic wave directly from the horn tip.

2.3.2. Ultrasound treatment with 40 kHz bath

Samples were placed in 100 ml flat bottom conical flask for ultrasound treatment with bath (40 kHz). Samples were treated for 15 and 30 min, where Erlenmeyer flask was immersed into a 40 kHz bath (Model SO375T, HF-Pk-power 300 W- overall dimensions: 370 × 175 × 250 mm; internal dimensions: 300 × 150 × 150 mm, Sonomatic, Warrington, UK). The treatment times were selected according to the significance of effect which was observed in preliminary research conducted in our Laboratory. An ultrasonic transducer was attached to the outer surface of the liquid container and the liquid was irradiated with an ultrasonic wave from the surface of the liquid container.

2.3.3. Ultrasound treatment with 500 kHz bath

Samples (100 ml) were placed in 250 ml Erlenmeyer conical flask for ultrasound treatment with high frequency bath (500 kHz). Samples were treated for 15 and 30 min with 500 kHz (512 kHz) bath (Model ES01/06/92, power 100 W, Undatim Ultrasonics S.A., Nivelles, Belgium).

2.3.4. Determination of ultrasound power and intensity

Ultrasonic power, which is considered as mechanical energy, would partly lose in the form of heat when ultrasound passes through the medium (Thompson and Doraiswamy, 1999). Since the ultrasonic irradiation of a liquid produces heat, recording the temperature as a function of time leads to the acoustic power estimation (in W) by the equation (Margulis and Malt'sev, 1969; Margulis and Margulis, 2003).

$$P = m \cdot c_p \cdot \left(\frac{dT}{dt}\right) \quad (1)$$

where: m – is the mass of the sonicated liquid (g), c_p – specific heat of medium at a constant pressure dependent on composition and volume of medium (J $(gK)^{-1}$), dT/dt – slope at the origin of the curve.

Ultrasound intensity is expressed in watts per unit area of the emitting surface, (W cm^{-2}), or in watts per unit volume of the sonicated solution (W cm^{-3}).

Ultrasonic intensity has been measured by calorimetry by thermocouple (model: HI 9063, Hanna Instruments Ltd. Leighton Buzzard LU7 4AD, UK) and expressed in W cm^{-2}.

2.4. Determination of electrical conductivity and temperature changes of soy protein model systems

Changes in electrical conductivity were determined using a calibrated PTI-8 Digital Electrical conductivity Meter (PTI-8 Digital Electrical conductivity Meter, Scientific Industries International Inc. UK) described in details elsewhere (Jambrak et al., 2008).

During ultrasound treatment temperature has been controlled by thermocouple, and the average temperature increase was expressed related to the room temperature (untreated sample-A) (model: HI 9063, Hanna Instruments Ltd., Leighton Buzzard LU7 4AD, UK).

2.5. Specific surface area and diameters determination

Specific surface area and diameters determination were carried directly after ultrasound treatment, when the particles were still dispersed in the collection fluid, by laser light scattering (Malvern Mastersizer 2000 equipped with a 100 mm lens, Malvern Instruments Limited, Malvern – Worcestershire, UK with Hydro MU sample dispersion unit).

2.6. Determination of soy proteins solubility

After ultrasound treatment soy protein were lyophilized in freeze dryer (Che ml ab Instruments Ltd., Hornchurch, Essex, UK; Model SB6CB) by freezing for a minimum of 3 h to temperature of −45 °C. Lyophilized protein powders were dispersed (1% w/w) in deionized water. The solubility of protein was determined at pH 7.0 by the method described by Smith et al. (1985). The concentration of proteins was determined using bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL, USA). Stock

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solutions (0.1% protein (w/w)) of control and treated soy proteins were prepared and allowed to hydrate overnight at 5 °C. Aliquots of the solutions (1 ml) were centrifuged at 12,500 g for 25 min at 20 °C. 100 μl of diluted supernatant sample (1/100) was added to 2 ml of BCA reagent. The tubes were incubated at 37 °C for 30 min and then cooled to 20 °C before measurement using a spectrophotometer at 562 nm (Helios ß, Unicam, UK). Protein content was determined after calibration using bovine serum albumin dilution series of five concentrations as external standard and the protein solubility was calculated as the percentage of soluble protein in the supernatant relative to the total protein content in the sample.

2.7. Foaming properties of soy protein model systems

For determination of foaming properties samples were prepared as described in Section 2.2 and then ultrasonically treated as described in Section 2.3. Foaming properties have not been measured for the following model systems because they have gelled during ultrasound treatment. For soy protein concentrate model systems after 20 kHz probe treatment for 15 min (B1), and for 40 kHz bath treatment for 15 min (C1). Then it could not been measured for soy protein isolates model system after 20 kHz probe treatment for 15 min (B1) because this system creamed during ultrasound treatment. After ultrasound treatment, suspensions were whipped at room temperature with blender (Morphy Richards Go Cordless Rechargeable Multi Tool, Argos, UK) equipped with a wire whip beater at maximum speed setting for up to 15 min to determine maximum foam expansion. Whipping was interrupted every 5 min during the run in order to determine foam expansion. Foam expansion was determined by level-filling a 100 ml plastic weighing boat with foam and then weighed. Foam expansion was calculated using the expression:

$$\text{Foam expansion}(\%) = \frac{\text{Unwhipped suspension wt(g)- foam wt(g)}}{\text{Unwhipped suspension wt(g)}} \times 100 \quad (2)$$

Foam stability was determined by transferring 100 ml of maximum expansion foam into a pyrex filter funnel with dimensions of 7.5 cm inner top diameter, 0.4 cm inner stem diameter and 7.0 cm stem length. A small plug of glass wool was placed in the top of the funnel stem to retain the foam but allow drainage of the liquid. The time required (min) for drainage of the entire foam was determined for index of foam stability (Morr and Foegeding, 1990).

2.8. Emulsifying properties of soy protein model systems

For emulsifying properties determination samples were prepared as described in Section 2.2 and then ultrasonically treated as described in Section 2.3. Emulsifying properties have not been measured for systems described in Section 2.7 because of previously mentioned reasons. After ultrasound treatment, protein suspensions were analyzed by the turbidometric technique for emulsion activity index (EAI) and emulsion stability index (ESI) as previously described (Webb et al., 2002). Emulsions were prepared with 3% protein dispersions (w/v) using 10 ml of sunflower oil (Sainsbury's Sunflower Oil, Sainsbury's Supermarket Ltd, London, UK), by mixing for 90 s in a blender. The absorbance of the diluted emulsions was measured by spectrophotometer (Unicam UV-4 UV–vis spectrophotometer, Spectronic Instruments Inc., Rochester, NY) at 500 nm in 1 cm path length cuvettes.

The emulsifying activity index (EAI) was determined by the turbidimetric method of Pearce and Kinsella (1978). The absorbance was read initially, after what turbidity and EAI were calculated using the following formula:

$$T = \frac{2.303 \cdot A}{I} \quad (3)$$

Where T = turbidity, A = absorbance at 500 nm and I = path length of cuvette (m).

The emulsion activity index (EAI) was then calculated as:

$$EAI = \frac{2 \cdot 2.303 \cdot D \cdot A}{I \cdot \Phi \cdot C \cdot 10,000} = \frac{2 \cdot T}{\Phi \cdot C}, \left(\frac{m^2}{g}\right) \quad (4)$$

where D = is the dilution factor, A = the absorbance at 500 nm, I = the path length of the cuvette (cm), φ = the volumetric fraction of oil; C = the weight of protein per unit volume of aqueous phase before the emulsion was formed (g ml $^{-1}$) and 10,000 the correction factor for square meters, T = turbidity (calculated from above equation).

For emulsion stability determination the emulsion were held at 4 °C for 24 h and reanalyzed for emulsion activity as described previously. An emulsion stability index was calculated by the following formula:

$$ESI = \frac{T \cdot \Delta t}{\Delta T} \quad (5)$$

where T = turbidity value at 0 h, ΔT = change in turbidity during 24 h period and Δt = time interval (24 h).

2.9. Determination of rheological properties of soy protein model systems

Torque measurements were carried out on the 10% (w/w) model dispersions using a Rheometric Viscometer (Model RM 180, Rheometric Scientific, Inc., Piscataway, USA) with the spindle (no. 3; Ø = 14 mm; l = 21 cm). Shear stress against the increasing shear rates from lowest value of 0–1290 s^{-1} as well as downwards was applied. Volume of the beaker was 36 ml. The samples were kept in a thermostatically controlled water bath for about 15 min before measurements in order to attain desirable temperature of 25 °C. Measurements were done in triplicates for each sample. The shear rate versus shear stress was interpreted using the Rheometric computer program. The values for n and k were obtained from plots of log shear stress versus log shear rate, according to the power law equation:

$$\log \tau = \log k + n \log \gamma \quad (6)$$

where τ is the shear stress (Pa); γ is the shear rate (s^{-1}); n is the flow behaviour index, and k is the consistency index (Pa s^n).

Apparent viscosity (η_{app}) was calculated at 1290 s^{-1} using Newtonian law, in addition with linear least square method for regression analysis.

$$\tau = \eta_{app} \gamma \quad (7)$$

2.10. Statistical analyses

The whole study was repeated and each value represents the mean of three measurements from three independent ultrasound treatments. The effect of ultrasound treatment on tested parameters was determined by analysis of variance, using statistical analyses with SPSS for Windows version 13.0 (SPSS Inc., Chicago, IL). Analysis of variance (One-Way ANOVA), significant level used was 5% (α = 0.05), was carried out to assess whether the different treatments conducted to statistically different results for those variables evaluated. The values not statistically different are accompanied by the letter (a) and the values statistically different with the letter (b).

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3. Results and discussion

3.1. Ultrasound intensity

In ultrasonic treatment with 20 kHz probe the ultrasonic intensity was 45–52 W cm^{-2}, in ultrasound treatment with 40 kHz bath the ultrasonic intensity was 2 W cm^{-2}, and for ultrasound treatment with 500 kHz bath the ultrasonic intensity did not exceed 1 W cm^{-2} as measured calorimetrically.

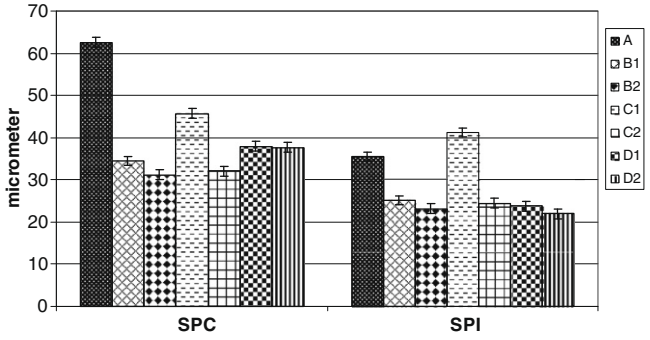
3.2. Ultrasound effect on changes in conductivity values and temperature changes of soy protein model systems

Results for electrical conductivity shows the changes in ion count in model systems after ultrasound treatment. Since cavitation is the main effect of sonication with ultrasound probe (less with ultrasound bath), there can be induced possible changes in total ion count. Table 1 shows the results of electrical conductivity after several ultrasound treatments. One may observe that there is significant increase ($p < 0.05$) in values of electrical conductivity after ultrasound treatment with 20 kHz probe for 15 min (B1). There are many reports, which have proved the formation of hydroxyl radicals during sonication (Petrier et al., 1992; Makino et al., 1983; Hart and Henglien, 1985). Possible increase in electrical conductivity is because of deterioration of ultrasound probe tip and small release of particles into model system. After other treatments with ultrasonic baths of 40 kHz and 500 kHz frequency there is decrease in electrical conductivity, except for one treatment (D2-500 kHz bath treatment for 30 min) for SPI sample but not statistically significant. This can be explained on the basis of the fact that the active cavity area in the case of bath is much more (surface area of US irradiating face of bath is 56× more then the surface area of the irradiating face of horn) then in case of horn. Also, the presence of ion aggregates that are not included in conductivity process and the increase in viscosity results in decreased electrical conductivity (Bohnke et al., 1993; Southall et al., 1996). Temperature of soy protein model systems increased significantly after ultrasound treatments having largest increase after ultrasound treatment with 20 kHz probe (23–42 °C and 45 °C, respectively), and the lowest increase after ultrasound treatment with 500 kHz bath (23–28 °C and 31 °C, respectively) (Table 1). Average increase in temperature is largest after ultrasound treatment with 20 kHz probe (21.1 °C).

Table 1
Values of pH, electrical conductivity (mS/cm) and temperature of samples (°C) before and after ultrasound treatment.

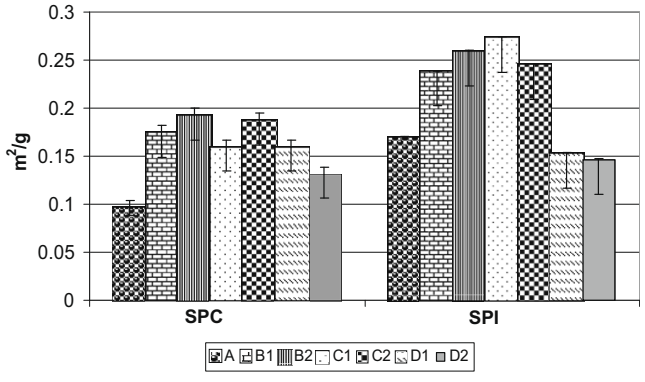
Treatment	Electrical conductivity (mS/cm)		Temperature (°C)	
	SPC	**SPI**	**SPC**	**SPI**
A	3.28 ± 0.12[a]	3.97 ± 0.09[a]	23 ± 0.1[a]	23 ± 0.1[a]
B1	4.11 ± 0.16[b]	4.90 ± 0.14[b]	42 ± 0.3[a]	42 ± 0.3[a]
B2	2.88 ± 0.21[b]	3.56 ± 0.12[b]	45 ± 0.4[a]	44 ± 0.2[a]
C1	3.13 ± 0.13[a]	3.62 ± 0.17[b]	29 ± 0.2[a]	29 ± 0.4[a]
C2	2.97 ± 0.14[b]	2.99 ± 0.18[b]	32 ± 0.4[a]	32 ± 0.2[a]
D1	2.51 ± 0.20[b]	2.97 ± 0.16[b]	28 ± 0.3[a]	30 ± 0.2[a]
D2	3.13 ± 0.18[a]	4.04 ± 0.12[a]	31 ± 0.1[a]	32 ± 0.1[a]

No ultrasound (**A**); 20 kHz probe – 15 min (**B1**); 20 kHz probe – 30 min (**B2**);
40 kHz bath – 15 min (**C1**); 40 kHz bath – 30 min (**C2**);
500 kHz bath – 15 min (**D1**); 500 kHz bath – 30 min (**D2**).
Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b) as compared to control.



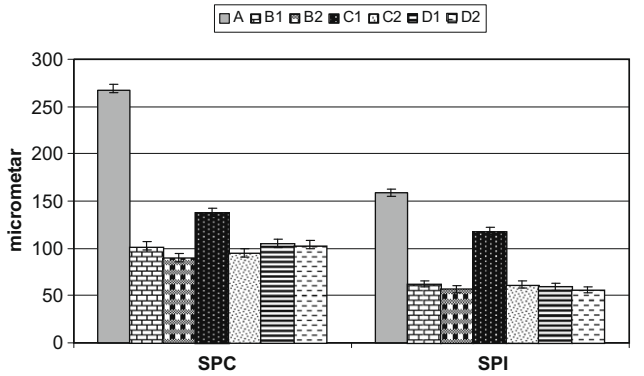
No ultrasound (**A**); 20 kHz probe – 15 min (**B1**); 20 kHz probe – 30 min (**B2**);
40 kHz bath – 15 min (**C1**); 40 kHz bath – 30 min (**C2**);
500 kHz bath – 15 min (**D1**); 500 kHz bath – 30 min (**D2**).

Fig. 2. Volume–surface average diameter D[3,2] of soy protein samples before and after ultrasound treatment.



No ultrasound (**A**); 20 kHz probe – 15 min (**B1**); 20 kHz probe – 30 min (**B2**);
40 kHz bath – 15 min (**C1**); 40 kHz bath – 30 min (**C2**);
500 kHz bath – 15 min (**D1**); 500 kHz bath – 30 min (**D2**).

Fig. 1. Specific surface area of soy protein samples before and after ultrasound treatment.



No ultrasound (**A**); 20 kHz probe – 15 min (**B1**); 20 kHz probe – 30 min (**B2**);
40 kHz bath – 15 min (**C1**); 40 kHz bath – 30 min (**C2**);
500 kHz bath – 15 min (**D1**); 500 kHz bath – 30 min (**D2**).

Fig. 3. Weight mean diameter D[4,3] of soy protein samples before and after ultrasound treatment.

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3.3. Influence of ultrasound treatment on specific surface area and diameters of soy proteins

The particle size reduction by the ultrasonic cavitation increases the surface area in contact between the solid (soy proteins) and the liquid phase (water), significantly (Fig. 1). This is the result of strong cavitational forces that are present in system during ultrasound treatment. From the results shown it could be seen that the largest increase in specific surface area for soy protein concentrate samples are after ultrasound treatment with 20 kHz probe for 30 min (B2) from 0.095 to 0.192 $m^2\ g^{-1}$, and for soy protein isolates after ultrasound treatment with 40 kHz bath (0.169–0.273 $m^2\ g^{-1}$).

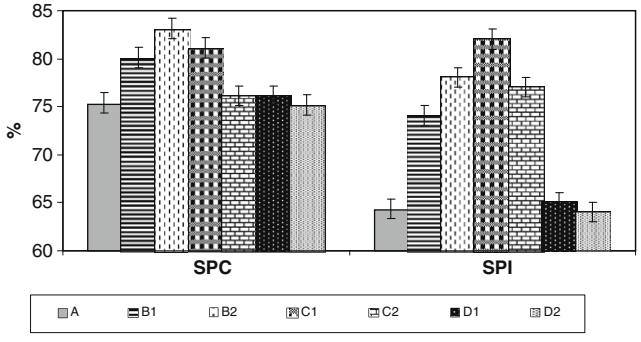
The values before and after ultrasound treatment of volume–surface average diameter D[3,2] of soy protein samples are shown in Fig. 2 and weight mean diameter D[4,3] of soy protein samples before and after ultrasound treatment are shown in Fig. 3. The Sauter mean diameter (SMD) or D[3,2] is defined as the diameter of a sphere that has the same volume/surface area ratio as a particle of interest. The mean droplet size was characterized in terms of the equivalent volume-weighted moment mean diameter (VMD) also known as the De Brouckere mean moment diameter D[4,3]. Results have shown the statistically significant ($p < 0.05$) decrease in average diameter. Its origin is in cavitational forces of ultrasound treatment with probe, and micro-streaming and turbulent forces after ultrasound treatment with baths. The largest decrease in average volume–surface diameter is after ultrasound treatment with 20 kHz bath for soy protein isolates where values are from 35.55 μm to 25.17 μm (20 kHz probe for 15 min-B1) and 23.13 μm (20 kHz probe for 30 min-B2). For soy protein concentrates there is larger initial value of volume surface diameter because of different composition having also carbohydrates and a fat in it's the composition. There is also the largest decrease in volume–surface average values after ultrasound treatment with probe for 30 min (62.55–31.18 μm) and 20 kHz probe treatment for 15 min (62.55–34.40 μm), respectively. It could be observed that for sample prepared with soy protein isolate there is also significant decrease in D[3,2] diameter with ultrasound baths, especially after ultrasound treatment with 500 kHz bath. This can be explained with the fact that soy protein isolate have mainly proteins in its composition and are very sensitive to elevated temperatures, which is in the case of ultrasound treatment as compared to concentrates where there are carbohydrates and fats that are showing some kind of protective effect for proteins when treated with ultrasound (Dumay et al., 1994).

3.4. Influence of ultrasound treatment on soy proteins solubility

Ultrasound treatment with 20 kHz probe has showed the largest increase in protein solubility ($p < 0.05$) of soy protein concentrates model systems as compared to untreated sample. Increase in solubility is greatest for 20 kHz treatment for 30 min (B2), followed by 40 kHz bath treatment for 15 min (C1). For other treatments there was not statistically significant change in protein solubility (Fig. 4). For soy protein isolate model systems it could be observed the largest increase in protein solubility after ultrasound treatment with 40 kHz bath for 15 min. Statistically significant increase in protein solubility for soy protein isolate model systems can be observed for 20 kHz probe treatment for both 15 and 30 min (64.3–74%, and 78%, respectively), and 40 kHz bath treatment for 15 and 30 min-C1 and C2 (64.3–78%, and 82%, respectively). No significant changes in soy protein solubility can be observed for 500 kHz bath treatments –D1 and D2 (Fig. 4). The main reason for the increase in protein solubility is in the fact that during ultrasound treatment large number of cavitation bubbles produces large increase in local temperature and pressure in the surrounding area of collapsing bubble which leads to unfolding of protein and braking of peptide bonds by hydrolysis. High intensity (probe-20 kHz) ultrasound increases protein solubility by changing protein conformation and structure so that hydrophilic amino acid residues are oriented toward water (Morel et al., 2000; Moulton and Wang, 1982). This treatment leads to decrease in molecular weight of proteins, whereas the larger area of proteins is covered with water molecules (Morel et al., 2000).

3.5. Influence of ultrasound treatment on foaming properties of soy proteins

In addition to its' functional properties soy proteins are used in food products because of their high proportion in the amino acids which decrease cholesterol and lower insulin levels. Some aspect of this study includes ultrasound treatment as a tool to prepare creams and gels that can be used as base for the development of other products. Results of ultrasound treatment with 20 kHz probe for 15 min showed for soy protein concentrates rapid gelling of sample after 3 or 4 min treatment (Table 2). The high increase in temperature is causing denaturation of soy proteins and leads to gelling (Bryant and McClements, 1998). Interestingly this kind of behaviour has not been shown for soy protein concentrate samples for 20 kHz probe treatment for 30 min (B2). The 20 kHz probe treatment of soy protein isolate is leading to formation of cream, and that happens around 5 min treatments. Other results are



No ultrasound (**A**); 20 kHz probe – 15 min (**B1**); 20 kHz probe – 30 min (**B2**);

40 kHz bath – 15 min (**C1**); 40 kHz bath – 30 min (**C2**);

500 kHz bath – 15 min (**D1**); 500 kHz bath – 30 min (**D2**).

Fig. 4. Solubility (%) of soy proteins before and after ultrasound treatment.

Table 2
Foaming properties: foam capacity (%) and foam stability FS (min) of soy protein samples before and after ultrasound treatment.

Foam capacity (%)			Foam stability FS (min)	
Treatment	**SPC**	**SPI**	**SPC**	**SPI**
A	95 ± 1.1[a]	110 ± 1.1[a]	61.5 ± 1.1[a]	55.1 ± 1.1[a]
B1	**Gelled**	**Creamed**	**Gelled**	**creamed**
B2	104 ± 1.3[b]	123 ± 1.3[b]	65.6 ± 1.0[b]	59.3 ± 1.2[b]
C1	**Gelled**	153 ± 1.2[b]	**Gelled**	62.5 ± 1.0[b]
C2	96 ± 1.3[a]	121 ± 1.2[b]	68.6 ± 1.2[b]	57.6 ± 1.0[a]
D1	93 ± 1.2[a]	107 ± 1.1[a]	60.8 ± 1.3[a]	55.3 ± 1.0[a]
D2	96 ± 1.1[a]	113 ± 1.1[a]	60.4 ± 1.4[a]	54.8 ± 0.9[a]

No ultrasound (**A**); 20 kHz probe – 15 min (**B1**); 20 kHz probe – 30 min (**B2**);
40 kHz bath – 15 min (**C1**); 40 kHz bath – 30 min (**C2**);
500 kHz bath – 15 min (**D1**); 500 kHz bath – 30 min (**D2**).
Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b) as compared to control.

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showing the significant increase in foam capacity (%) for soy protein isolate samples after 20 kHz probe treatment for 30 min (B2) (110–123%) and 40 kHz bath treatments for 15 (C1) (to 153%) and 30 min (C2) (to 121%). Also, significant increase in foam capacity for soy protein concentrate samples can be observed for 20 kHz probe treatment for 30 min (95–104%). There were no other significant changes in foam capacity for other treatments (Table 2). The most stable foams are shown for ultrasound treatment with ultrasound 40 kHz bath for soy protein concentrate where increase is (61.5–68.6 min) for 30 min treatment (C2), and for soy protein isolate where increase was (55.1–62.5 min) for 15 min treatment (C1) as compared to untreated one.

3.6. Influence of ultrasound treatment on emulsifying properties of soy proteins

Ultrasound treatment of soy proteins alters their emulsifying properties. The major alterations induced by the treatment are a decrease of the droplet size. Soy proteins exhibit partial unfolding of 7S and 11S fractions, and an aggregation of proteins, especially of the 11S fraction. Partial denaturation and a more disordered structure are able to provide a better potentiality for the adsorption at the oil–water interface. These results are shown as increase in emulsion activity indices (EAI) for both soy proteins isolate and concentrate model systems (Table 3). This could be explained by the decrease of droplet size and the increase of the percentage of adsorbed proteins as the ultrasound treatment is improved (Caessens et al., 1999). Under turbulent conditions that are occurring during ultrasound treatment (like homogenisation) movements favour the adsorption of proteins and formation of aggregates predominates (Walstra, 1983). We can assume that the soluble aggregates generated by ultrasound treatment are likely to be adsorbed at the oil–water interface. One can suggest that this increase leads to a better potential adsorption of proteins at the oil–water interface. Other results showed significant increase in EAI for ultrasound treatment with probe, 40 and 500 kHz baths. These emulsions have been prepared after ultrasound treatments of model systems where soy protein are opened are reoriented toward oil–water interface. The increase of emulsion stability index (ESI) could be explained by better orientation of proteins, under the influence of turbulent behaviour produced by ultrasound, and integration of oil bubbles in the emulsion.

Table 3
Emulsifying properties: emulsion activity index - EAI (m^2/g) and emulsion stability index - ESI (h) of soy protein samples before and after ultrasound treatment.

Emulsion activity index - EAI (m^2/g)			Emulsion stability index - ESI (h)	
Treatment	**SPC**	**SPI**	**SPC**	**SPI**
A	121.35 ± 1.12^a	110.20 ± 1.72^a	75.3 ± 1.33^a	54.3 ± 1.14^a
B1	**Gelled**	**Creamed**	**Gelled**	**Creamed**
B2	277.54 ± 1.45^b	184.56 ± 1.12^b	145.2 ± 1.21^b	145.2 ± 1.25^b
C1	**Gelled**	165.67 ± 1.63^b	**Gelled**	132.3 ± 1.36^b
C2	265.78 ± 1.13^b	157.84 ± 1.34^b	159.4 ± 1.23^b	150.7 ± 1.72^b
D1	212.35 ± 1.54^b	145.12 ± 1.28^b	165.4 ± 1.14^b	78.4 ± 1.23^b
D2	214.67 ± 1.71^b	143.89 ± 1.19^b	156.7 ± 1.18^b	75.4 ± 1.20^b

No ultrasound (**A**); 20 kHz probe – 15 min (**B1**); 20 kHz probe – 30 min (**B2**);
40 kHz bath – 15 min (**C1**); 40 kHz bath – 30 min (**C2**);
500 kHz bath – 15 min (**D1**); 500 kHz bath – 30 min (**D2**).
Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b) as compared to control.

3.7. Influence of ultrasound treatment on rheological properties of soy protein model systems

Rheological properties of foods are very important because in food processing one have to know the exact behaviour of foods for development and for plant composition in order to produce specific product. The ultrasound treatment besides it cavitational effect by which it effects different functional properties of soy protein suspensions, possess microbiological destruction effect and changes in flow behaviour of liquid food. This kind of behaviour has been shown in this study where ultrasound treatment caused statistically significant ($p < 0.05$) changes in flow behaviour indices (n) and consistency coefficients (k). Rheological properties of soy protein isolates suspensions have been influenced in greater extend by ultrasound treatment and the largest increase in consistency coefficients (k) have been observed after ultrasound treatment with 40 kHz bath for 30 min (C2) (46.4–5337 · 10^3 mPa s), and for soy protein concentrates (76.5–1766 · 10^3 mPa s) (Table 4). The flow behaviour indices show the pseudoplastic ($n < 1$) behaviour, and it remains pseudoplastic after ultrasound treatments. A pseudoplastic material is one in which viscosity decreases with increasing rate of shear (also termed shear thinning). Shear rate and shear stress relationship of untreated and treated

Table 4
Apparent viscosity (η_{app}), flow behaviour indices (n), consistency coefficients (k) and regression coefficients of untreated and SPC/SPI treated with ultrasound.

	Treatment	Apparent viscosity (η_{app}) mPa s	Consistency coefficients (k) mPa s	Flow behaviour indices (n)	Regression coefficients (r^2)
SPI	A	16.0 ± 0.1^a	46.4 ± 0.1^a	0.853 ± 0.235^a	0.998
	B1	71.0 ± 0.2^b	1.3 ± 0.2^b	0.313 ± 0.032^b	0.994
	B2	177.0 ± 0.3^b	$38\ 10^3 \pm 1.1^b$	0.245 ± 0.011^b	0.996
	C1	246.0 ± 0.2^b	$2063\ 10^3 \pm 1.2^b$	0.263 ± 0.036^b	0.990
	C2	222.0 ± 0.1^b	$5337\ 10^3 \pm 1.4^b$	0.447 ± 0.016^b	0.985
	D1	296.0 ± 0.3^b	$3887\ 10^3 \pm 1.6^b$	0.319 ± 0.062^b	0.991
	D2	276.0 ± 0.1^b	$2573\ 10^3 \pm 1.5^b$	0.239 ± 0.052^b	0.991
SPC	A	15.0 ± 0.1^a	76.5 ± 0.1^a	0.769 ± 0.132^a	0.997
	B1	586 ± 0.2^b	$1665\ 10^3 \pm 1.3^b$	0.134 ± 0.215^b	0.991
	B2	556 ± 0.3^b	$1706\ 10^3 \pm 1.4^b$	0.147 ± 0.236^b	0.991
	C1	375 ± 0.2^b	$1138\ 10^3 \pm 1.3^b$	0.139 ± 0.040^b	0.996
	C2	436 ± 0.2^b	$1766\ 10^3 \pm 1.1^b$	0.158 ± 0.052^b	0.991
	D1	354 ± 0.1^b	$832\ 10^3 \pm 1.2^b$	0.081 ± 0.103^b	0.995
	D2	324 ± 0.1^b	$820\ 10^3 \pm 1.1^b$	0.096 ± 0.104^b	0.938

●At 1290 s^{-1}.
No ultrasound (**A**); 20 kHz probe – 15 min (**B1**); 20 kHz probe – 30 min (**B2**);
40 kHz bath – 15 min (**C1**); 40 kHz bath – 30 min (**C2**);
500 kHz bath – 15 min (**D1**); 500 kHz bath – 30 min (**D2**).
Each value represents the mean of three measurements from three independent ultrasound treatments. The values not statistically different are accompanied by the same letter (a) and the values statistically different with another letter (b) as compared to control.

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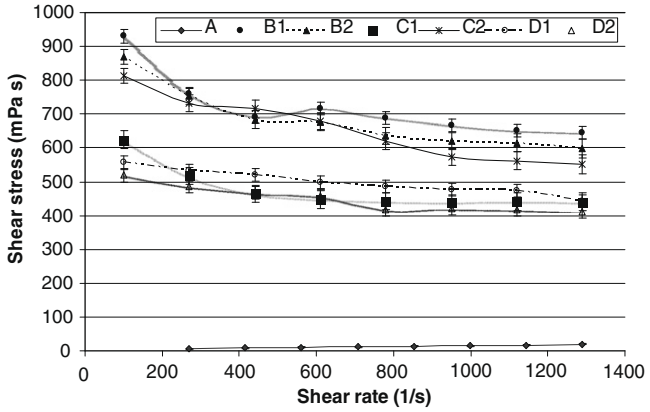
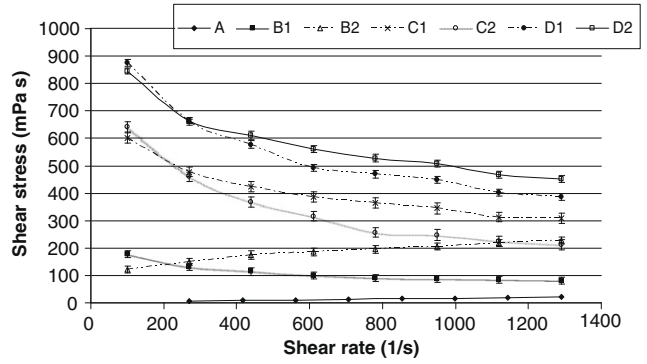


No ultrasound (**A**); 20 kHz probe – 15 min (**B1**); 20 kHz probe – 30 min (**B2**);

40 kHz bath – 15 min (**C1**); 40 kHz bath – 30 min (**C2**);

500 kHz bath – 15 min (**D1**); 500 kHz bath – 30 min (**D2**).

Fig. 5. Shear rate and shear stress relationship of untreated and treated soy protein concentrate (SPC) with ultrasound.



No ultrasound (**A**); 20 kHz probe – 15 min (**B1**); 20 kHz probe – 30 min (**B2**);

40 kHz bath – 15 min (**C1**); 40 kHz bath – 30 min (**C2**);

500 kHz bath – 15 min (**D1**); 500 kHz bath – 30 min (**D2**).

Fig. 6. Shear rate and shear stress relationship of untreated and treated soy protein isolate (SPI) with ultrasound.

soy protein isolate (SPI) and soy protein concentrates (SPC) with ultrasound are shown in Figs. 5 and 6. This property is also a common property of polymer solutions and molten polymers. From the results shown before, we can conclude that during ultrasound treatment there was rapid molecule movement due to cavitation (probe) and microstreaming (bath) and unfolding of protein chains, leading to hydroxyl radical development of water hydrolysis (Hart and Henglien, 1985) and radical chain reaction leading to polymerization. The final obtained product or soy protein concentrates after specific ultrasound treatments (20 kHz/15 min-B1 and 40 kHz-15 min – C1) was gel, and for soy protein isolate after ultrasound treatment with 20 kHz probe for 15 min (B1) was cream.

4. Conclusions

Ultrasound treatment presents possible method of producing soy products and creams as compared to traditional production. Application of ultrasound is less energy consuming and is time effective as compared to traditional and current technology. Nowadays, soy products are produced and processed like any product usually made with dairy milk.

In this study ultrasound treatment caused significant changes in texture of model systems prepared with soy protein concentrates, that gelled during ultrasound treatment with probe 20 and bath 40 kHz for 15 min. Model system prepared with soy protein isolate (SPI) creamed during ultrasound treatment with probe 20 kHz for 15 min. Ultrasound treatment with 20 kHz probe caused significant changes in conductivity, increased solubility for soy protein concentrates, significantly increased specific surface area that is of interest in food texture and increased values of emulsion activity index (EAI). Weight mean diameter D[4,3] and volume–surface average diameter D[3,2] decreased significantly for all samples and all treatments. Rheological properties of soy protein isolates and soy protein concentrates suspensions have been greater influenced by ultrasound treatment and the largest increase in consistency coefficients (k) have been observed after ultrasound treatment with 40 kHz bath. The flow behaviour indices show the pseudoplastic ($n < 1$) behaviour, and it remains pseudoplastic after ultrasound treatment. There was no improvement in foaming or emulsifying properties of soy protein model systems after 500 kHz bath treatment.

There are number of possible new product concepts that can be produced using soy proteins as a base and with combination with ultrasound like: smoothies, cheese alternatives, soy cream based soups, spreads and creamy dressings. The main precaution that needs to be taken care when using ultrasound is to conduct process that is not going to elevate temperature because of highly temperature sensitive soy proteins.

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References

Bohnke, O., Frand, G., Rezrazi, M., Rousselot, C., Truche, C., 1993. Fast ion transport in new lithium electrolytes gelled with PMMA. 2. Influence of lithium salt concentration. Solid State Ionics 66, 105.

Bryant, C., McClements, D., 1998. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat denatured whey. Trends in Food Science Technology 9, 143–151.

Burrington, K., 2000. Nutritional and Beneficial Ingredients. Food Product Design, 1–12.

Caessens, P.W., De Jongh, H.H., Norde, W., Gruppen, H., 1999. The adsorption-induced secondary structure of beta-casein and of distinct parts of its sequence in relation to foam and emulsion properties. Biochimica et Biophysica Acta 1430, 73–83.

Dumay, E.M., Kalichevsky, M.T., Cheftel, J.C., 1994. High pressure unfolding and aggregation of β-lactoglobulin and the baroprotective effects of sucrose. Journal of Agriculture and Food Chemistry 42, 1861–1868.

Entezari, M.H., Hagh Nazary, S., Haddad Khodaparast, M.H., 2004. The direct effect of ultrasound on the extraction of date syrup and its micro-organisms. Ultrasonics Sonochemistry 11 (6), 379–384.

Guzey, D., Gulseren, I., Bruce, B., Weiss, J., 2006. Interfacial properties and structural conformation of thermosonicated bovine serum albumin. Food Hydrocolloids 20, 669–677.

Hart, E.J., Henglien, A., 1985. Free radical and free atom reactions in the sonolysis of aqueous iodide and formate solutions. Journal of Physical Chemistry 89, 4342–4347.

Jambrak, A.R., Mason, T.J., Lelas, V., Herceg, Z., Herceg, L.J.I., 2008. Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. Journal of Food Engineering 86 (2), 281–287.

Leadley, C., Williams, A., 2002. Power ultrasound–current and potential applications for food processing, Review No 32, Campden and Chorleywood Food Research Association.

Makino, K., Mossoba, M.M., Riesz, P., 1983. Chemical effects of ultrasound an aqueous solutions. Formation of hydroxyl radicals and hydrogen atoms. Journal of Physical Chemistry 87, 1369.

Margulis, M.A., Malt'sev, A.N., 1969. Russ. J. Phys. Chem. 43, 055 (Translation of Zh.Fiz. Khim).

Margulis, M.A., Margulis, I.M., 2003. Calorimetric method for measurement of acoustic power absorbed in a volume of a liquid. Ultrasonic Sonochemistry 10, 343–345.

Mason, T.J., 1990. A general introduction to sonochemistry. In: Mason, T.J. (Ed.), Sonochemistry: The Uses of Ultrasound in Chemistry. The Royal Society of Chemistry, Cambridge, UK, pp. 1–8.

Mason, T.J., 1998. Power ultrasound in food processing—the way forward. In: Povey, M.J.W., Mason, T.J. (Eds.), Ultrasound in Food Processing. Thomson Science, London, UK, pp. 22–25.

McClements, J., 1995. Advances in the application of ultrasound in food analysis and processing. Trends in Food Science and Technology 6, 293–299.

Morel, M.-H., Dehlon, P., Autran, J.C., Leygue, J.P., Bar-L'Helgouac'h, C., 2000. Effects of temperature, sonication time, and power settings on size distribution and extractability of total wheat flour proteins as determined by size-exclusion high-performance liquid chromatography. Cereal Chemistry 77, 685–691.

Moulton, K.J., Wang, L.C., 1982. A pilot-plant study of continuous ultrasonic extraction of soybean protein. Journal of Food Science 47, 1127–1129.

Morr, C.V., Foegeding, E.A., 1990. Composition and functionality of commercial whey and milk protein concentrated and isolates: a status report. Food Technology 44 (4), 100–112.

Nielsen, M., 1997. Texture measurement of fruits and vegetables by the use of ultrasonics. PhD thesis, The Royal Veterinary and Agricultural University, Copenhagen.

Orthoefer, F.T., 1978. Processing and utilization. In: Soybean: Physiology, Agronomy, and Utilization, pp. 219–246.

Pearce, K.N., Kinsella, J.E., 1978. Emulsifying properties of proteins: evaluation of a turbidometric technique. Journal of Agricultural and Food Chemistry 26, 716–723.

Petrier, C., Jeunet, A., Luche, J.L., Reverdy, G., 1992. Unexpected frequency effects on the rate of oxidative processes induced by ultrasound. Journal of the American Chemical Society 114, 3148.

Richert, S.J., Kolar, C.W., 1987. Value of isolated soy protein in food products. In: Cereals and Legumes in the Food Supply. Iowa State University Press, Ames, IA, pp. 73–90.

Russell, T.A., 2004. Comparison of sensory properties of whey and soy protein concentrates and isolates. Thesis for Master of Science, North Carolina State University, USA.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using bicinchoninic acid. Analytical Biochemistry 150, 70–76.

Thompson, L.H., Doraiswamy, L.K., 1999. Sonochemistry: science and engineering. Industry Engineering and Chemistry Research 38, 1215–1249.

Southall, J.P., Hubbard, H.V.StA., Johnston, S.F., Rogers, V., Davies, G.R., McIntyre, J.E., Ward, I.M., 1996. Ionic conductivity and viscosity correlations in liquid electrolytes for incorporation into PVDF gel electrolytes. Solid State Ionics 85, 51.

Soy Protein Council (1987). Soy Protein Products, Characteristics, Nutritional Aspects and Utilization. Soy Protein Council, Washington DC.

Suslick, K.S., 1988. Ultrasound: its chemical, physical, and biological effects. In: Suslick, K.S. (Ed.), Ultrasound. VCH Press, New York.

Walstra, P., 1983. Formation of emulsion. In: Becher, P. (Ed.), Encyclopedia of Emulsion Technology: Basic Theory, vol. 1. Marcel Deckker, New York, pp. 57–127.

Wang, Q., Tolkach, A., Kulozik, U., 2008. Quantitative assessment of thermal denaturation of bovine alpha-lactalbumin via low-intensity ultrasound, HPLC, and DSC. Journal of agricultural and food chemistry 54 (18), 6501–6506.

Webb, M.F., Naeem, H.A., Schmidt, K.A., 2002. Food protein functionality in a liquid system: a comparison of deamidated wheat protein with dairy and soy proteins. Journal of Food Science 67, 2896–2902.

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