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Physicochemical Properties of Soy Protein Concentrate Treated with Ultrasound at Various Amplitudes

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ABSTRACT: This research was conducted to investigate the influence of ultrasound amplitude on the physicochemical properties of soy protein concentrate. Soy protein concentrates (SPC, Acron SM) were treated with a frequency of 20 kHz ultrasound and three different amplitudes of 50, 80, and 100% for 5 min. Untreated and ultrasound-treated soy protein concentrate samples were evaluated in terms of recovery of soluble protein, particle size, surface hydrophobicity, free sulfhydryl groups, turbidity and microstructure. The environmental scanning electron microscope images of the treated and untreated soy protein concentrate samples were taken in order to analyze the microstructure of the samples. The findings showed that the ultrasound treatment have a significant effect on all physicochemical characteristics ($p<0.05$). All ultrasound treated samples showed significantly higher solubility compared to the untreated soy protein concentrates. In addition, the highest protein solubility was determined for the samples treated with 100% amplitude. Ultrasound treatment reduced the size of all proteins. The sample which has the highest solubility also showed the lowest particle size compared to the others. Moreover, ultrasound treated (100% amplitude) soy protein concentrate was resulted with highest surface hydrophobicity and free sulfhydryl groups. Microscope images of the soy protein concentrates showed a spherical morphology with particle diameters which closely corresponding to the results obtained by dynamic light scattering. It was clearly seen that increasing ultrasound amplitude enhance the functionality of soy protein concentrates.

Keywords: Ultrasound amplitude, soy protein concentrate, particle size, protein solubility, surface hydrophobicity, free sulfhydryl group.

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INTRODUCTION

Soy protein is a heterogeneous mixture of storage proteins, which are generally classified through their separation by using centrifugal sedimentation in sucrose gradients. There are four major fractions in soy protein, which are classified in relation to their sedimentation coefficients, 2S, 7S, 11S, and 15S (Kinsella, 1979; Liu, 1997). Two major soy proteins, glycinin (11S) and b-conglycinin (7S), affect the processing functionality of soy protein ingredients. These two proteins have a similar structure as both of them derived from X-ray crystallography (Moreira et al., 1979; Adachi., 2003). Whole soybean is processed into several products including roasted soy nuts, soy flour, defatted flakes, soy protein concentrates (SPC), and soy protein isolates (SPI) (Liu, 1997). Soy protein concentrate is a soy product containing at least 65% protein but less than 90% protein. Soy protein concentrates are produced by removing soluble sugars, ash, and minor components from the 50% protein soy flour starting material. The protein is insolubilized, and soluble components are washed out (Campbell et al., 1985). Even though there is a growing interest in using soy proteins because of several advantages compared to other sourced proteins such as high nutritional value, steady supply, and cheap in recent years, soy proteins as emulsifiers are usually reported to be less effective rather than other proteins, such as casein (Santiago et al., 1998). This might be because of the compact structures of soy proteins that stabilized mostly by hydrogen bonds and disulfide bonds (Palazolo et al., 2005). Several methods have been developed in order to change the native structure of soy proteins to enhance the functionality. For instance, supposing the proteins to very acidic or alkaline pH values (Molina et al., 2001; Puppo et al., 2005). Modified soy protein concentrates demonstrate a very high degree of functionality. Through mechanical and/or chemical modifications it is possible to rearrange protein molecules so that they become more functional. A modified soy protein concentrate described by Howard et al. (1980) showed an increase in its water solubility. This product exhibited functionality in various meat systems which was better than compared to soy protein isolates, and so it might be replaced with milk proteins at lower cost in several applications (Moore et al., 1980).

High intensity ultrasound (HUS) is a cost-effective and quick technology which has been used in order to change the structural and functional characteristics of proteins (Mason et al., 1996; Jambrak et al., 2008). The effect of ultrasound (US) is achieved by the chemical, mechanical, and physical effects of acoustic cavitation.

The cavitation basically described as a formation, growth, and violent collapse of small bubbles in liquid. The cavitation might lead to modification of protein structure with the help of hydrogen bonds and hydrophobic interactions, and breaking down protein aggregates (Mason et al., 1996) By considering the advantages such as being a cost-effective, non-toxic, quick and effective technology, it is expected to achieve improved SPC functionality by using US treatment. Therefore, the aim of this study is to examine the effect of US treatment on the physical and chemical properties of soy protein concentrate.

MATERIALS AND METHODS

Material

SPC (Acron SM) was supplied from Archer Daniels Midland Company (ADM, Decatur, IL, USA) and it consists of 69% soy protein on dry base.

Sample preparation and ultrasound treatment

Ultrasound (US) treatment was conducted using a VC-750 ultrasound power supply with the frequency of 20 kHz (Sonics & Materials, Inc., USA) and three different amplitudes of 50, 80, and 100% for 5 min. Insoluble SPC (3 g) was mixed with a 100 mL distilled water and stirred during half an hour at room temperature (RT) with a magnetic stirrer. The beaker was placed in an ice bath at the time of sonication to avoid the temperature increase. The protein dispersions after the ultrasound treatment were centrifuged (1,200 g and 20^{o}C) during 15 min. Soluble SPC was obtained after the centrifugation step. For the control samples, no US treatment was applied, 3 g SPC in 100 mL was stirred at RT during half an hour. Table 1 shows the explanation of the samples and treatments.

Table 1. The explanation of the samples and treatments

Treatments	Stirring	Ultrasound (50% amplitude)	Ultrasound (80%)	Ultrasound (100%)	Centrifuge
Control	1	2	2	2	1
US5*	1	1	2	2	1
US8	1	2	1	2	1
US10	1	2	2	1	1

*Control: "Untreated SPC, no ultrasound"; US5 stands for "Ultrasound treatment with 50% amplitude"; US8 stands for "Ultrasound treatment with 80% amplitude", and US10 stands for "Ultrasound treatment with 100% amplitude"
(1: shows the steps applied; and 2: shows the steps that were not applied)

Solubility

Solubility of the samples was measured according to the method proposed by Bradford (1976). Bovine serum albumin (BSA) was used as the standard. Dye reagent was prepared by diluting 1 part of dye reagent with 4 parts of distilled water, and filtered through a filter paper (0.22 mm pore size, 13 mm diameter, PTFE syringe filter, Whatman, Piscataway, NJ, USA). This dilution was mixed with soluble SPC. Protein concentration of soluble SPC was determined by spectrophotometer at 595 nm. The solubility was calculated as Equation 1 and expressed as "%":

$$Recovery\ of\ soluble\ protein\ (\%) = \frac{\boldsymbol{Protein\ concentration\ in\ soluble\ SPC}}{\boldsymbol{Initial\ protein\ concentration}} \times 100 \quad (1)$$

Surface hydrophobicity

Surface hydrophobicity (H_0) of SPC dispersions was determined according to the method of Yildiz et al. (2017). 1-anilino-8-naphthalenesulfonate (ANS) was used as the fluorescence probe. ANS stock solution (8 mM) was prepared in phosphate buffer (0.01 M, pH 7). Similarly, different soy protein concentrations, changes from 0.04 to 0.2 mg mL^{-1}, were prepared with same phosphate buffer (0.01 M, pH7). ANS stock solution (20 µL) was mixed with protein solutions and the intensity was measured at 340 nm (excitation) and 440 nm (emission). The slope of fluorescence intensity vs. protein concentration were calculated and referred as H_0 of proteins.

Free sulfhydryl groups

Free sulfhydryl groups (Free-SH) were determined as proposed by Lee et al. (2016). A cysteine hydrochloride monohydrate (changing from 0 to 1.5 Mm) was dissolved in a sodium phosphate buffer (0.1. M). 50 µL of Ellman's reagent solution was added in the mix which consist of 250 µL of protein sample and 2.5 mL of sodium phosphate buffer. The solution was well-mixed and after incubation at RT for 15 min, the absorbance at 412 nm was measured. The free SH content of SPC samples was expressed as µmol g^{-1}.

Particle size and turbidity

Particle sizes of the SPC samples were determined by dynamic light scattering (DLS) using a NICOMP 380 DLS instrument. Samples were diluted 500-fold with distilled water prior to analysis. All measurements were performed at RT. The average of 3 runs was used to calculate particle size (nm).

Turbidity of the SPC solutions was determined with a spectrophotometer following method stated by Yildiz et al. (2017). Distilled water was used as the blank, and the absorbance at 600 nm was read.

Environmental scanning electron microscope (ESEM)

The morphology of the SPC samples was analyzed by Environmental Scanning Electron Microscope (ESEM). The SPC samples were tested under wet mode.

The sample was frozen with nitrogen prior to ESEM analysis. Small amount of frozen sample was taken into an aluminum stub and was put into vacuum chamber. The samples were analyzed by the microscope (ESEM, Philips XL30 ESEM-FEG, FEI Co., U.S.A.) with the voltage of 5.0 kV.

Statistics

The differences were determined by using the General Linear Models procedure in SAS (version 9.3, SAS Institute, Inc., Cary, North Carolina, USA). Significant differences among the means were identified with Fisher's least significant difference (LSD) test at alpha = 0.05.

RESULTS AND DISCUSSION

Solubility

Table 2 shows the recovery of soluble protein (%) of SPC samples treated with ultrasound. The highest protein solubility was observed in the US treated SPC with 100% amplitude (51.15%), while the lowest protein solubility (1.37%) was observed in the untreated SPC. In addition, US treated SPC with 50% and 80% amplitudes showed significantly higher protein solubility compared to the control. However, these two has significantly lower soluble protein than that of ultrasound treated SPC with 100% amplitude. Higher amplitudes led to higher solubility. Several studies reported that the improvement of soy protein solubility after a US treatment (Lee et al., 2016; Yildiz et al., 2017). Yildiz et al. (2017) observed that the solubility of the soy protein isolates (SPI) was significantly increased with the Mano-thermo-sonication (MTS) treatment which is the most powerful ultrasonication type. It was stated that the MTS treated SPI increased solubility from 9.08 % for the control to 82.5 %. Similarly, it was achieved in the study of Lee et al. (2016) significantly higher protein solubility of soy proteins treated with ultrasound and pH shifting process. In another study, a modified soy protein concentrate showed an increase in its water solubility (Howard et al., 1980). The physical forces created by ultrasonic cavitation such as shear forces might change the structure of proteins which results in improved protein solubility. In addition, sonication may also break the non-covalent and covalent bonds which leads to SPI solubility increase (Hu et al., 2003).

Surface hydrophobicity

Surface hydrophobicity (H_0) values of the SPC samples are shown in Table 2. While the lowest Ho (142.0) was found for the Control samples, the highest H_0 (198.0) was found for the ultrasound-treated SPC solutions with 100%. There are not any significant changes were determined between the SPC samples treated with ultrasound at 50% and 80% amplitudes ($p>0.05$). A positive relationship was determined between solubility and Ho (Table 2). For example, the US-treated SPC (100%) samples showed the highest solubility (51.15%) which had also the highest Ho (198.0). In a similar way, the control samples showed the lowest solubility (1.37%), and its Ho (142.0) was also the lowest. This finding is supported by the observation of Yildiz et al. (2017) who found a positive linkage between the solubility and Ho of soy protein isolates. It was also confirmed by the work of Lee et al. (2016) and Jiang et al (2017).

Both Ho and solubility are the major parameters which affects the emulsifying activity of a protein (jiang et al., 2011). Good emulsifying and foaming ability is the result of balance between hydrophilic and hydrophobic groups (Jambrak et al., 2008) The US-treated SPC showed both high solubility and increased surface hydrophobicity, which might be an indicator of better emulsifying capacity and stability.

Free sulfhydryl groups

Free SH groups of untreated and US-treated SPC samples are displayed in Table 2. The free SH contents of the US-treated SPC samples at 100% were the highest among all other treatments (4.05 μmol g^{-1}). The lowest SH contents are observed in the Control (3.64 μmol/g). There is no significant difference was found between the US5 and US8 samples ($p>0.05$) (Table 2). A higher SH content shows mainly exposure of internal SH groups because of the protein unfolding caused by ultrasonic cavitation. Hence, the surface SH content depends on the conformation changes and protein unfolding (Jiang et al., 2017). The increase in free SH content could also be caused by smaller SPC particle sizes after ultrasound treatment, which causes the buried SH groups in SPC to be supposed to the surface. An increase in free SH contents also reported by Lee et al. (2016) and Yildiz et al. (2017) in the US treated soy protein samples compared to the control. In addition, US10- treated SPC sample had the highest protein solubility (51.15%) which shows the increase in SH content contributed to increase solubility.

Table 2. Protein solubility (% recovery), surface hydrophobicity, and free-SH groups of SPC samples

Treatments	Solubility (%)	Surface hydrophobicity (H_0)	Free SH (µmol g^{-1})
Untreated SPC	1.37 ± 0.23^{c}	127 + 0.3^{c}	3.64 + 0.22^{c}
US5	38.40 ± 0.39^{b}	170 + 0.5^{b}	3.83 + 0.14^{b}
US8	43.22 ± 0.28^{b}	172 + 0.7^{b}	3.88 + 0.68^{b}
US10	51.15 ± 0.22^{a}	198 + 0.4^{a}	4.05 + 0.76^{a}

$^{a-c}$ Mean ± standard deviation (n=3) of properties with the same letter are not significantly different ($p < 0.05$)
*All the statistics were done separately for each parameters (solubility, surface hydrophobicity, and Free-SH)

Particle size and turbidity

DLS results of the soluble SPC aggregates for 3 treatments are tabulated in Table 3. The largest particle size was observed for the control (293.4 nm). On the other hand, the smallest particle size was found for the SPC treated with 100% amplitude (93.5 nm) which is less than 100 nm. SPC samples treated with 50% and 80% amplitudes also showed the smaller particle sizes compared to the untreated SPC. However, their sizes were significantly larger than US10 treatment. A reduction in the particle size of plant proteins was achieved by using ultrasound technology in several studies. When used in protein dispersions, sonication was reported to significantly reduce the particle sizes of SPC (Jambrak et al., 2009). In addition, Karki et al. (2010) observed that the particle size of defatted soy flakes was reduced nearly 10-fold by ultrasonic treatment. It was reported that the cavitation might be reason of breakage of soy protein aggregates, and reduction in particle sizes (Arzeni et al., 2012).

Both the number of soluble soy protein aggregates in the solution determined by solubility and the sizes of the soluble protein aggregates determines the turbidity of a SPC solution. (Gregory,1998). The turbidity results of SPC samples are displayed in Table 2, and the appearance of the untreated and ultrasound treated SPC (with a 100% amplitude) is shown in Figure 1. The US10 samples had high number concentration (solubility), however since they also showed the smallest particle sizes, their turbidity was found as the lowest (0.35). The sample showed almost transparent appearance (Figure 1). On the other hand, the control looked cloudy as it showed the largest particle sizes (293.4 nm) (Table 3).

Table 3. Particle size (nm) and turbidity of SPC samples

Treatments	Particle size (nm)	Turbidity
Untreated SPC	293.4 ± 2^{a}	1.04 ± 0.2^{a}
US5	148.2 ± 1^{b}	0.94 ± 0.3^{a}
US8	127.7 ± 3^{bc}	0.44 ± 0.1^{b}
US10	93.5 ± 2^{c}	0.35 ± 0.1^{b}

$^{a-c}$ Mean ± standard deviation (n=3) of properties with the same letter are not significantly different ($p < 0.05$)
*All the statistics were done separately for each parameters (solubility, surface hydrophobicity, and Free-SH)

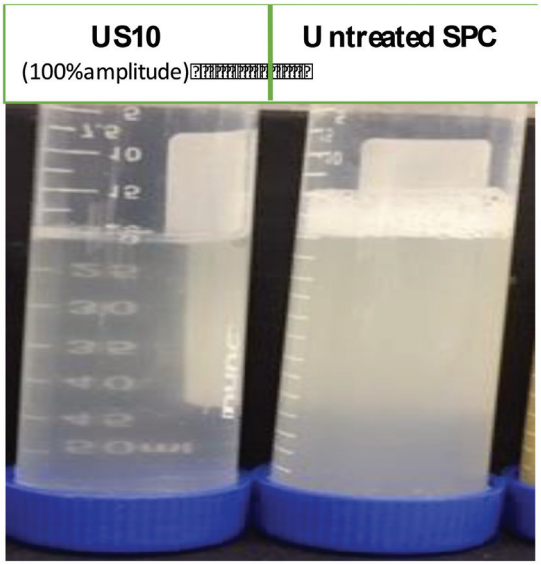
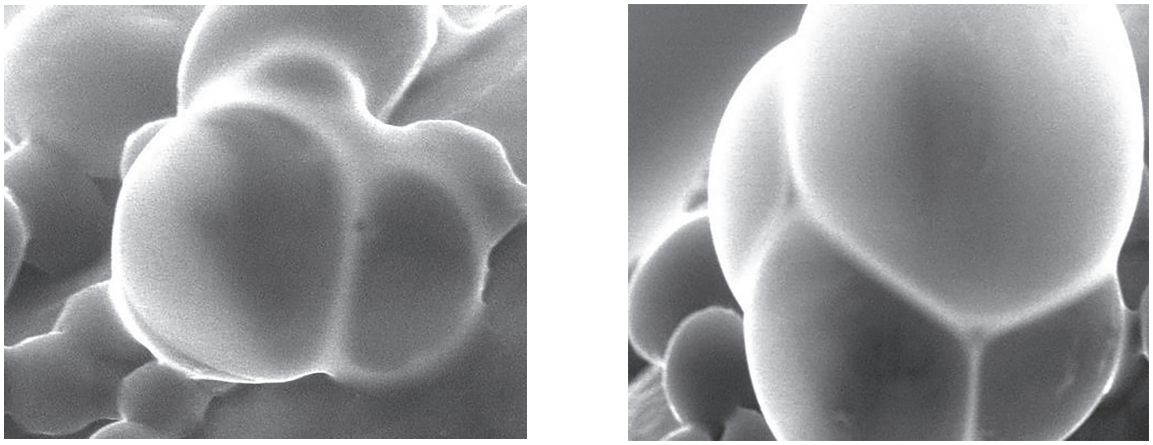


Figure 1. The appearance of control and US-treated SPC with a 100% amplitude

Environmental scanning electron microscope (esem)

ESEM images (500 nm) of the control and US-treated SPC (100% amplitude) samples are shown in Figure 2. Both SPC samples exhibited a spherical morphology with particle diameters closely corresponding to the results obtained by DLS (Table 3).



US10 **Untreated SPC**

Figure 2. ESEM images of the untreated and US-treated SPC with a 100% amplitude

CONCLUSION

The findings show that the ultrasound treatment specifically with higher amplitudes significantly increased the solubility of soy protein concentrates, and reduced the sizes of protein aggregates to less than 100 nm. Overall, ultrasound treatment is a promising method to enhance the functional properties of soy proteins as shown in this study by its ability to higher solubility, H_0, free SH groups and smaller particle size right after ultrasonication compared to the untreated samples. In overall, soy protein concentrates produced by ultrasound treatment can be used as a wall material for encapsulation of bioactive compounds in order to produce plant protein-based food products with improved properties.

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