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ENGINEERING, ENVIRONMENT AND MATERIALS IN PROCESSING INDUSTRY

PROCEEDINGS

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ENHANCING PROTEIN RELEASE AND FUNCTIONALITY OF SOY PROTEINS FROM DEFATTED SOY FLAKES USING HIGH-INTENSITY ULTRASOUND-ASSISTED EXTRACTION

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Abstract

In addition to evidence regarding their potential for inclusion into functional foods, the effect of the high-intensity ultrasound extraction (HIUE) on the functional properties of soy proteins will be discussed. The aim of this study was to examine the effects of HIUE as a function of the treatment time and ultrasound amplitude, in order to obtain better understanding of physicochemical effects of HIUE on soy protein which may lead to improving its applications in the food industry.

For this purpose, samples of the yellow defatted soybean (Glycine max) variety Laura were treated with ultrasound probe system (20±0.2 kHz) by varying the treatment time (30 s, 2, 5, 10, 15 min) and an amplitude (15% and 30%). Functional properties of the obtained samples were characterized in terms of solubility and sulfhydryl content. HIUE resulted in a slight, but gradual decline in solubility with a treatment time, but significant increase ($p < 0.05$) in acidic environment was observed with the increase of ultrasound amplitude. Sulfhydryl content has been greater influenced with a treatment time for both amplitude applied.

The results show that the ultrasound extracted soy proteins were superior to the original (untreated) in the functional properties tested and can be concluded that by combining the treatment time and the ultrasonic power, the samples with enhanced functionalities can be produced enhancing utilization of soy proteins in food products.

Keywords: *soy proteins, ultrasound probe extraction, solubility, sulfhydryl content*

Introduction

Soybean (*Glycine max*) is a protein-rich leguminous oilseed which contains abundant high quality proteins, since its proteins have high biological value while is cost is relatively low. Given the potential health benefits of food protein, there is a growing interest in developing protein fortified foodstuffs. One of these is that some soybean protein products with special functional properties are available in market, so improvement in functional properties of soybean protein products may further increase their applications in foodstuffs.

Soy proteins (SPs) have been widely used in processing foods as an important ingredient due to their highly nutritious and desirable functional properties. It is known that soybean proteins have several physiological functions such as cholesterol-lowering and body-fat reducing effects which maintain the role of soybean proteins in reducing the risk of coronary heart disease [1,2]. Nevertheless, in many cases, the application of soy proteins is limited due to incompatibility between their solubility and other functional properties. To accomplish desirable properties, many physical, chemical and enzymatic modifications have been applied to soy proteins. Lately, the application of high-intensity ultrasound treatment (HIUT) to modify the properties of food proteins has become an area of considerable research interest and investigations have been presented by many researches [3-5]. Some of the research studies have investigated the application of HIUT during protein chemical reaction or as pretreatment, so as to promote the subsequent modification. For instance, Mu et al. (2010) reported that ultrasound treatment was an efficient method for forming protein and polysaccharide conjugates, while Chen, Chen, Ren, and Zhao (2011) reported on the use of ultrasound pretreatment to increase the enzymatic hydrolysis of protein [6,7]. Other studies have applied HIUT to directly modify the functional and physical properties of proteins, such as solubility, gelation, emulsification and foamability. Solubility and foaming ability of a-lactalbumin and egg white proteins were improved by HIUT [8,3].

However, to the best of our knowledge the potential of HIUT to enhance protein release and their functional properties from defatted soy flakes has not been clearly established. Because the release of protein is governed by disintegration of lamellar structures binding the protein molecules, it is possible to hypothesize that the use of HIUT improves protein release and ultimately alter solubility and others functional properties. Thus, the purpose

of this study was to investigate the effects of HIUT on the functional properties of soy protein, as a function of the ultrasound amplitude (15% and 30%) and time (30 s, 2, 5, 10 and 15 min) of treatment, in order to obtain a better understanding of physicochemical effects of HIUT on soy protein which may lead to improving its applications in the food industry.

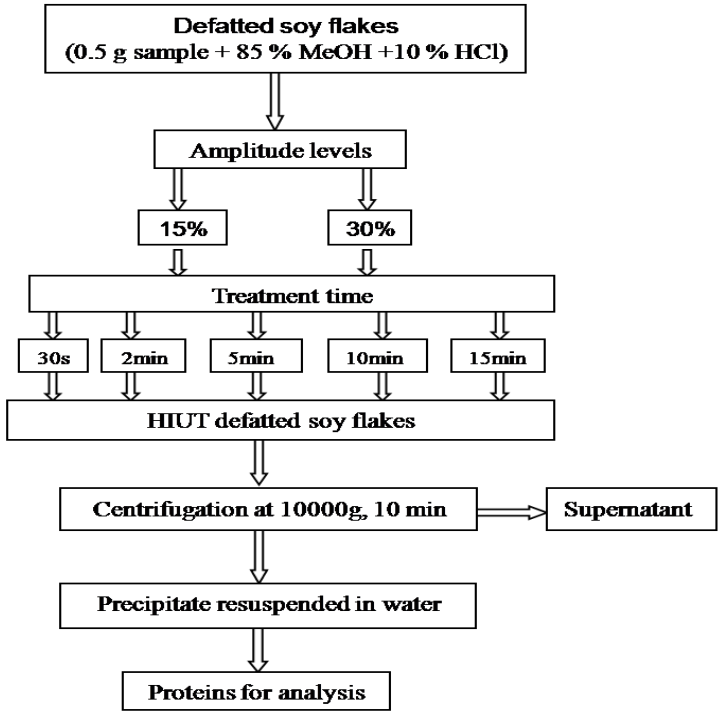
Materials and methods

Material

Yellow soybean varieties Laura used for the purpose of this study was obtained in experiments conducted in 2013 and 2014 in Zemun Polje, on calcareous chernozem goods, on a micro-assay (dimension of the parcel was 5x5 m with three experimental replicates per treatment). Samples of soybeans after the completion of the harvest, were cleaned of residual pods and impurities, milled and passed through a laboratory sieve hole diameter of 5mm. Thereafter, the samples were frozen at -20 °C and stored in the freezer until analysis. Other reagents were obtained from Sigma Aldrich, St. Louis, USA and were analytical grade.

Sample preparation

Defatted soy flakes (0.5 g) were dispersed in 10 ml solvent (85% MeOH and 10% HCl) in a customized stainless steel sonication vessel with a cooling water jacket. These samples were sonicated using a probe-type sonicator at a frequency of 20±0.2 kHz at two different amplitude levels (15 and 30%). For each amplitude, the samples were sonicated during 30s, 2min, 5min, 10min and 15 min with temperature moderation. A 13 mm high grade titanium tip was immersed in the samples and the liquid is irradiated with an ultrasonic wave directly from the horn tip. The power levels were changed by varying the amplitude amplitude at the horn tip. The constant temperature (25±1 °C) was maintained by circulating thermostated water through a water jacket (ordinary water flow: from 5 to 10 mL/s). A schematic of sonication extraction procedure is shown in Scheme 1.



Scheme 1. Schematic representation of an experimental procedure

Protein solubility of soy proteins

To determine protein solubility of ultrasound treated SPs, 0.2 ml of the sample was taken in 20 ml of distilled water and pH of the mixture was adjusted to 2, 4, 6, 8, 10 and 12 with 0.5 M HCl or 0.5 M NaOH. The mixture was stirred at room temperature for 1h and centrifuged at 12,000×*g* for 10 min. Protein contents in the supernatant were determined using the Lowry method with BSA as the standard. Solubility was expressed as the percentage of protein remaining in the supernatant as compared to the total protein content in the sample after solubilization.

Determination of sulfhydryl (SH) content

The content of SH groups of SPs upon treatment with the ultrasound probe was determined spectrophotometrically using 5,5′-(dithiobis-2-nitrobenzoate), DTNB, which reacted with free SH groups to yield a product with a maximum absorbance at 412 nm. Analysis was performed according to Ellman's procedure with slight modifications [9]. The reactive (surface) SH groups were measured as follows. A solution of sonicated SPs or control was diluted to a concentration of 0.05 % (w/w) with a standard buffer, pH

8.0 composed of 86 mM TRIS, 90 mM glycine, 4 mM EDTA and centrifuged during 20 min at 7889×*g* to remove precipitated proteins. A 0.025 mL of Ellman's reagent (4 mg/mL) was added to 2.5 mL of supernatants, mixed rapidly and after 15 min at ambient temperature, the absorbance was measured at 412 nm against a reagent blank. The total SH groups' content was also determined following the same technique, but using a denaturing buffer consisting of 86 mM TRIS, 8 M urea and 0.5 % (w/v) sodium dodecyl sulphate. The standard and the denaturant buffers were used as reagent blanks instead of protein solutions for both measurements.

Results and discussion

High intensity ultrasound-assisted extraction of soy proteins

The protein enhancing from defatted soy flakes using high-intensity ultrasound probe was examined according to the effect of two different ultrasonication conditions. First was the input power expressed as 15 and 30% amplitude levels, and second was the ultrasonication time (30s and 2, 5, 10 and 15 min). The main purpose of these both experimental sets was to optimization of ultrasonication conditions for maximum recovery of soy proteins. The values of the extracted protein yield (%) calculated from the measured protein content in the resuspended precipitate relative to the total protein content of defatted flakes are presented on Figure 1.

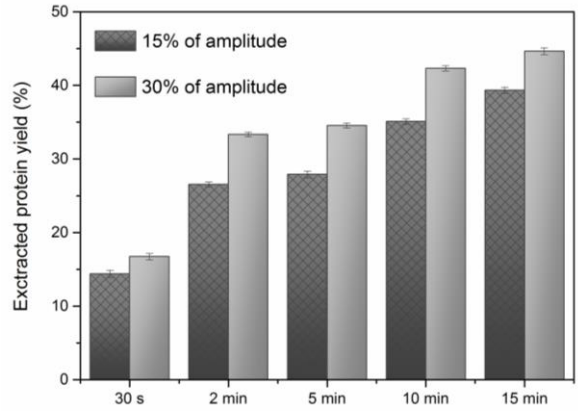


Figure 1. The extraction protein yield at various ultrasonication conditions (extraction conditions were as follows: MeOH:HCl=85:15, 5% (w/w) solution of defatted soy flakes). The extracted protein yield for control (non-sonicated deffated soy sample) was 7%.

Some differences between the extracted protein yield of all defatted soy samples depending on the used time and the amplitude of the ultrasound sonication were observed. After 30 s of ultrasound-assisted extraction at both used amplitude, the protein release increased with respect to the control by

twofold. It is apparent that the defatted soy samples after 15 min ultrasound extraction with both amplitude levels generated more extracted proteins than other ultrasonication times. More specifically, during the 15 min of ultrasonication with amplitude 15 and 30%, the resulting precipitates were enriched with proteins in the amount of 40 and 45%, respectively. These presented results were in a close agreement with that of *Karki et al. (2010)* [10] who were observed that treatment at high amplitude for 120 s gave the highest increase in protein yield, which was 46%, when compared to the control. Having regard to the results can be emphasized that different ultrasound-assisted extraction conditions seemed to significantly influence the increase of protein yield, which could affect the functional properties of the isolated proteins.

Effect of ultrasound-assisted extraction on the isolated protein solubility profile

Protein solubility profile represented a major functional property indicating how easy protein product can be incorporated into food formulation. The solubility often affects other functional properties and it's known that the lowest solubility of soy proteins is around pH 2-4. For these reasons, the solubility profile of isolated protein was determined according to the influence of amplitude levels and time of ultrasound extraction. The isolated protein solubility profiles are demonstrated on Figure 2.

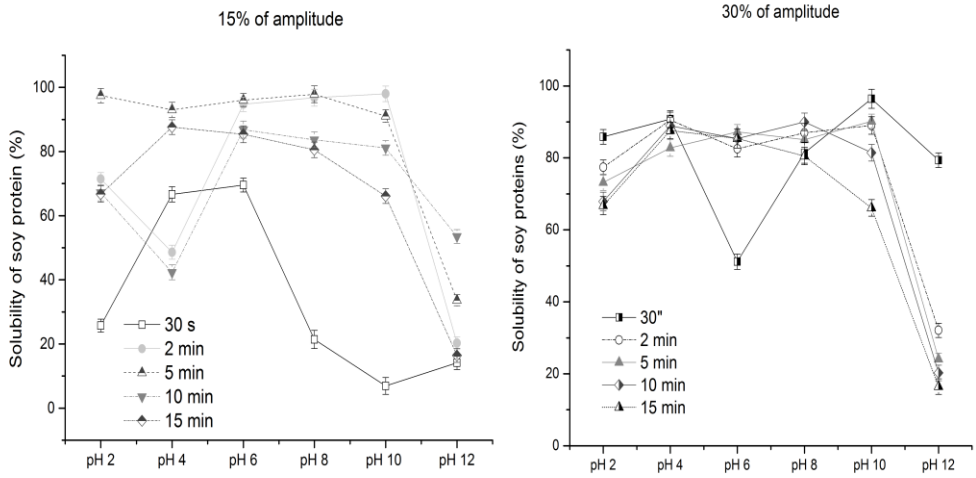


Figure 2. Solubility profiles of the isolated soy proteins according to the influence of amplitude levels and time of ultrasound extraction.

Generally, the ultrasound treatment favored the extraction more soluble soy proteins, especially on the pH range 4-10 for amplitude level 30%. In particular, can be emphasized that the minimum solubility of the soy sample

treated for 30 seconds was at pH 6 (isoelectric point), but for all other soy samples the lowest value of solubility moves in a very basic environment at pH 12. There were no statistically significant differences in solubility with the extension of the time ultrasonication at pH range 4 to 8. Only the sample of 30 s showed a different trend, suggesting that ultrasonication with increasing time isoelectric point is moved to the base environments. Solubility in the acidic environment has increased, but the negative trend with increasing treatment time was observed. These results are very interesting according to the technological aspects, because in the food industry generally tends to increase the solubility of soy protein in an acidic environment.

As shown on Figure 2, the solubility of the soy protein extracted with ultrasound treatment and amplitude level of 15%, the solubility increased compared to the shortest treated sample. Also, can be emphasized that in an acidic environment at pH 2, significantly increased solubility of the protein, which is essential for the application of soy protein in the food industry where they often work in acidic conditions. The largest and most stable value of solubility is present when ultrasound-assisted extraction was performed for 5 min. For pH values between 2 and 10 the solubility values was 90%, but for a distinctly base environment the solubility is low, as in all other cases. The highest ultrasoinication time led to the significant fluctuations in the results depending on the pH value.

Generally, from the accompanying figures can be showed that the largest and most stable solubility is present in the sample ultrasonicated over a period of 5 minutes, with the values of the solubility of 70% up to 98%, except for pH greater than 10. The analysis of this chart it can be seen that the better solubility (range 90% to 98%) was realized by ultrasound-extracted samples with amplitude level 15%. The presented results are in the contrast with results obtained by *Karki et al*. (2009) who also performed extraction defatted soy flakes with application ultrasound treatment and were obtained the typical U-shaped solubility curves of soy proteins with the lowest solubility at the isoelectric pH (4 to 5) [11].

According to the literature data, the main reason for the increased solubility of ultrasonicated soy proteins lies in the fact that during sonication a large number of cavitation bubbles lead to an increase in temperature and pressure around the decaying bubble and consequently, to the unwinding proteins and cleavage of intermolecular and peptide bonds. High-intensity ultrasound enhances the solubility of protein by changing their structure and conformation, so that the hydrophilic amino acid residues are oriented toward the water [12].

Effect of ultrasound-assisted extraction on the isolated protein structure

The influence of high-intensity ultrasound-assisted extraction on the isolates protein structure was characterized in terms of content total and exposed sulfhydryl groups by using the Ellman's reagent. The contents of total and exposed groups, expressed as μmol per g of proteins, depending on the effect of amplitude levels and time of ultrasound extraction are represented in Figure 3.

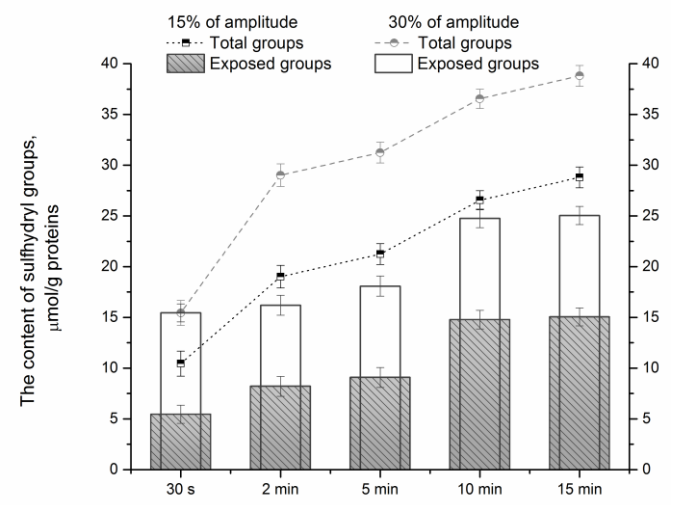


Figure 3. The effect of ultrasound-assisted extraction on the isolated protein structure in terms of content total and exposed sulfhydryl groups according to the influence of amplitude levels and time of ultrasound extraction.

According to the results represented in the Figure 3, could be observed that with the extension of ultrasound-assisted extraction time there is an increase the content of total and exposed sulfhydryl groups. Based on the above, it can be concluded that the released ultrasonic energy by the ultrasonic transducer frequency 20 kHz has led to changes in the molecular structure of isolated proteins. This can be attributed to oxidation of the SH groups to disulfide bonds (SS) during the process of ultrasonic extraction. Also, could be noted that the total and reactive sulfhydryl (SH) groups increased after extraction with higher amplitude level (30%).

Namely, after ultrasound extraction with amplitude 30% the sample treated for 30 seconds containing 15.4 μmol/g of total SH group, but the sample treated for 2 minutes even contains 29.1 μmol/g of total SH groups. Something smaller difference was occurred during the extraction with amplitude 15% where the content was changed from 10.4 to 19.1 μmol/g of total SH. Subsequent increase in ultrasound extraction time the content of total and exposed SH groups increases, but not so significantly.

Increasing the amplitude of ultrasonication has led to an increase in the content SH groups can be interpreted in that fact that higher amplitude of ultrasound leads to more intense breaking S-S bounds, or the reduction S-S bonds in S-H bonds. The glycinin (11S protein) contains at least 20 S-S bound connections within their molecules that can be reduced the influence of ultrasound and allow the formation of S-H bonds. Commenting on the available literature data, there were examples of breaking internal disulfide bonds soy protein isolates after sonication, but the results obtained in presented research were a lot better for both ultrasound amplitude. Thus, *Hu et al.* (2013) [13] did research with soy protein isolate, varying the power of ultrasound from 200 to 600 W and the time from 15 to 30 min. The results showed that with increasing time and power of ultrasound, the contents of SH groups were growing, which is consistent with demonstrated results. However, the highest content of SH group (18.08±0.39 μmol/g) was achieved after 30 min of ultrasonication with power of 600 W. The results obtained in this research proved to be much better, because for half time of sonication (15 min) and very lower power received values up to two times higher compared to the literature (38.81 μmol/g).

Conclusion

The main purpose of this research was to enhance the protein release and functionality of soy proteins from defatted soy flakes using high-intensity ultrasound-assisted extraction. In terms of mention, the high-intensity ultrasound-assisted extraction was conducted with various conditions: amplitude levels and time of ultrasound extraction. Functional properties of the extracted soy proteins were characterized in terms of protein yield, solubility and sulfhydryl content. Ultrasound-assisted extraction resulted in a slight, but gradual decline in solubility with an extraction time, but significant increase ($p < 0.05$) in acidic environment was observed with the increase of ultrasound amplitude. Sulfhydryl content has been greater influenced with a treatment time for both amplitude applied.

In conclusion, under the conditions investigated in this research, high-intensity ultrasound-assisted extraction resulted in partial unfolding and reduction of intermolecular interactions as demonstrated by increases in sulfhydryl groups, leading to improved solubility. The results show that the ultrasound-extracted soy proteins were superior to the non-sonicated deffated soy sample in the functional properties tested and can be concluded that by combining the ultrasonication time and an amplitude, the samples with enhanced functionalities can be produced enhancing utilization of soy proteins in food products.

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