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Structural and functional modification of proteins from black soybean Aquasoya via ultrasonication

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ABSTRACT

Plant-based proteins obtained from agricultural by-products have garnered growing interest in response to consumer awareness of health and environmental issues. This study aimed to improve the functionalities of the proteins recovered from black soybean Aquasoya (PBSA) by modifying their structure via ultrasonication. PBSA was ultrasonicated with a frequency of 40 kHz at 350 W for different time periods (0, 20, 40, and 60 min), and its structural characteristics, physicochemical properties, and functional properties were investigated. Ultrasonication left the primary structure intact but altered the secondary and tertiary structures of the PBSA; α-helix and β-sheet contents decreased, random coil contents increased, and buried non-polar amino acid residues were exposed. Moreover, ultrasound promoted an increase in free sulfhydryl content and a reduction in particle size. Consequently, functional properties, such as solubility, emulsion stability, and foaming performance were improved by modifying the structure and physicochemical properties of PBSA. This work demonstrates the potential of ultrasound, which is favorable for modifying the spatial conformation and related functionalities of proteins, thus meeting the needs of manufacturers to use function-enhanced proteins as food additives.

1. Introduction

Recently, there has been increasing interest in adding value to agro-industrial waste, especially because the global production of such waste amounts to approximately 190 million tons per year. Importantly, this waste also contains valuable compounds such as proteins, carbohydrates, and bioactive compounds [1,2]. In particular, the demand for proteins in the agricultural and food sectors has increased as the global population has grown, and plant-based proteins are attracting special attention because of increasing consumer awareness about health and environmental issues [3]. Therefore, on this basis, recovery of plant proteins from by-products has become an indispensable global challenge for the environment, economy, and society.

A promising source of potential protein currently discarded as waste is *Aquasoya*, the thick liquid produced when soybeans are boiled in water [1,4,5]. During soybean processing, including the manufacturing of tofu, soy milk, and soy sauce, a large volume of wastewater is produced and subsequently discarded, resulting in environmental pollution. However, Aquasoya contains nutrients such as proteins and polysaccharides, as well as small molecules that are eluted during boiling; thus, this liquid could prove to be a novel, clean, and sustainable source of protein. As shown by our previous work, the excellent emulsifying and foaming properties of Aquasoya make it suitable for use in vegan foods as an egg replacement in, for example, mayonnaise, meringues, and muffins [6–8].

Proteins not only act as macronutrients but also can be used as stabilizers because of their amphiphilicity, making them excellent techno-functional compounds. The structure of proteins strongly affects their functional properties, such as solubility and emulsifying ability. Therefore, many strategies have been developed to change protein structure [9]. Among these strategies, ultrasonic treatment is particularly efficient, allowing the modification of the structure, physicochemical properties, and functional properties of proteins. In particular, ultrasonic treatment is a non-thermal, low-cost, and environmentally friendly technique. The key mechanism of ultrasonic treatment is cavitation, which releases a large amount of energy as a result of the generation and rapid collapse of bubbles during treatment [10].

Recently, several studies have investigated ultrasonic treatment of proteins, especially with regard to increasing functional properties. In one study, the foaming capacity, surface hydrophobicity (H_0), and

Abbreviations: PBSA, Proteins from black soybean Aquasoya.

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solubility of jackfruit protein were improved by 15 min of high-intensity ultrasound treatment (200, 400, and 600 W) [11]. In addition, the use of an ultrasound intensity of 37 W/cm^3 was found to enhance the emulsification, oil absorption, foaming, and H_0 of hemp seed protein isolates [12]. Later, Martínez-Velasco et al. [13] reported that faba bean protein isolates treated ultrasonically showed low interfacial tension, ζ-potential, viscosity, enhanced solubility, and foaming properties compared to the untreated control.

However, investigation of the effects of ultrasonication on proteins from the cooking liquid of black soybeans, Aquasoya (PBSA) has been limited. Therefore, in this study, we aimed to investigate the relationship between ultrasonic treatment time and the structures and functional properties of PBSA. Briefly, the PBSA was recovered and sonicated for different periods (0, 20, 40, and 60 min). Subsequently, the changes in the primary, secondary, and tertiary structures, physicochemical properties (H_0, free sulfhydryl (SH) content, particle size, and ζ-potential) and functional properties (solubility, emulsifying properties, adsorbed protein (AP) content, and foaming properties), were examined.

2. Materials and methods

2.1. Materials

Black soybean Aquasoya was prepared following the method described in [1]. The beans were cooked under pressure for 160 min at a 2:3 (*w*/w) bean-to-water ratio, and the resulting Aquasoya was collected and frozen until use. Tris (hydroxymethyl) aminomethane was purchased from Daejung Chemicals and Metals Co. (Korea). Acetic acid, ammonium persulfate, hydrochloric acid, dibasic sodium phosphate, and monobasic sodium phosphate were purchased from Samchun Chemical Co. (Korea). 1-Anilino-8-naphthalene sulfonic acid (ANS), Coomassie Brilliant Blue, 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), ethylenediaminetetraacetic acid (EDTA), glycerol, β-mercaptoethanol, sodium dodecyl sulfate (SDS), and NNNN-tetramethylethylenediamine were obtained from Sigma-Aldrich Co. (USA). Acrylamide. bromophenol blue, glycine, and urea were acquired from Junsei Chemical Co. Ltd. (Japan). Bisacrylamide was obtained from Alfa Aesar Co., Inc. (USA).

2.2. Methods

2.2.1. Protein preparation

Proteins were recovered from black soybean Aquasoya through acid precipitation where Aquasoya (500 ml), thawed at 4 °C, was adjusted to a pH of 4.5 using 1 M HCl, followed by centrifugation at 4000 ×*g* for 15 min. The residue was washed three times with 400 ml distilled water (DW). The washed precipitate was then suspended in 200 ml DW, and the slurry was adjusted to a pH of 7.0. The dispersion was then stirred for 10 min and lyophilized using a freeze-dryer (FDS8508, IlshinBioBase, Korea). The dried cube was ground with a pestle and mortar and processed using a 200 μm sieve. The protein content of the PBSA powder was 46.25 ± 0.57%.

2.2.2. Ultrasound treatment

PBSA was dissolved in DW until a concentration of 30 mg/ml was achieved. An aliquot (50 ml) of the suspension was transferred into a 150 ml bottle and screw-capped. Ultrasonication was performed according to the method described previously with a minor modification [14]. The suspensions were sonicated for 20, 40, and 60 min in an ultrasound water bath (Power Sonic 505, HWASHIN, Seoul, Korea; internal dimensions: 300 × 155 × 150 mm^3) with a frequency of 40 kHz at 350 W, where ice was added repeatedly to prevent excessive temperature rise. Subsequently, all samples were freeze-dried, ground with pestle and mortar, and processed using a 200 μm sieve. The powder was packed in silica gel and kept at 4 °C for later use.

2.2.3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was carried out as described previously [15]. A 15% running gel and 4% stacking gel were used. Diluted samples (10 mg/ml) were added to a sample buffer and incubated at 95 °C for 5 min. An aliquot (20 μl) of the sample was loaded, and electrophoresis was conducted on the sample at 150 V. Coomassie Brilliant Blue was used for protein identification.

2.2.4. Secondary structure

A circular dichroism (CD) spectrometer (Chirascan plus, Applied-Photophysics, UK) was used to obtain CD spectra following the modified method described in [16]. Samples were diluted 40-fold with 0.01 M phosphate buffer solution (PBS) (pH 7.0) and placed in quartz cuvettes. Wavelengths ranging from 190 nm to 250 nm were measured. The secondary structures of the samples were interpreted using the BeStSel software (https://bestsel.elte.hu/index.php).

2.2.5. Tertiary structure

Fluorescence spectra were acquired as described in [17] using QuantaMaster™ 400 (HORIBA Scientific, Japan). The emission wavelength ranging from 300 to 500 nm was recorded with an excitation wavelength of 280 nm.

2.2.6. H_0

ANS was used as a fluorescence probe to determine H_0 using the method reported in [18]. Samples were diluted with 0.01 M PBS (pH 7.0) to protein concentrations ranging from 0.005 mg/ml to 0.03 mg/ml. Then, 20 μl of 8 mM ANS was mixed with 2 ml sample suspensions. The fluorescence intensity (FI) was detected at 470 nm (emission wavelength) with an excitation wavelength of 390 nm using QuantaMaster™ 400 (HORIBA Scientific, Japan). The FI was plotted as a function of protein concentration and the initial slope was calculated as H_0.

2.2.7. Particle size and distribution

The particle size and polydispersity index (PDI) of the samples (10 mg/ml) were determined using an ELSZ-1000 (Otsuka Electronics, Japan) following the method described in [19].

2.2.8. Free SH contents

The free SH content of the PBSA was determined according to the method described in [20]. Samples were dissolved in a buffer (86 mM Tris-90 mM glycine, 4 mM EDTA, pH 8.0) at a concentration of 2 mg/ml. Sample suspension (2 ml) was mixed with 20 μl of DTNB solution (4 mg/ml) and incubated for 1 h at 4 °C. The absorbance at 412 nm was recorded and the free SH content was calculated using a molar absorption of 13,600 l/mol·cm.

2.2.9. Solubility

Protein solubility was measured as described in [17] with slight modifications, where 100 mg samples were dissolved in DW (10 ml) and stirred for 20 min. The sample underwent centrifugation at 4000 ×*g* for 15 min after which the precipitate was dried at 106 °C overnight. The solubility was determined using the following equation:

$$Solubility\left(g/g\right) = \frac{powder\ weight - dry\ pellet\ weight}{powder\ weight} \quad (1)$$

2.2.10. Emulsifying properties

Emulsions were produced using the method described in [21]. The samples (7.5 ml of samples (10 mg/ml)) and corn oil (2.5 ml) were homogenized at 10,000 rpm for 1 min using an Ultraturrax T25, IKA. Emulsions (10 μl) were withdrawn from the bottom after 10 min and diluted 100-fold with a 0.1% (*w*/*v*) SDS solution. Subsequently, the absorbance was measured at 500 nm using a spectrophotometer

(Genesys 10S, Thermo Fisher). The emulsifying ability index (EAI) and emulsifying stability index (ESI) were calculated using the following equations:

$$EAI\ (m^2/g) = \frac{2 \times 2.303 \times D \times A_0}{C \times \Phi \times 10^4} \quad (2)$$

$$ESI\ (min) = \frac{A_0}{A_0 - A_{10}} \times 10 \quad (3)$$

where D is the dilution factor, A_0 and A_{10} are the absorbance at 0 and 10 min, respectively, C is the protein concentration (g/ml), and Φ is the oil volume fraction of the emulsion.

2.2.11. AP content

The emulsions prepared in Section 2.2.10 were centrifuged, followed by separating the aqueous phase with a syringe. The Bradford assay was conducted to evaluate the unadsorbed protein content. The AP content was calculated using Eq. (4) as follows:

$$APs\ content\ (\%) = \frac{initial\ protein\ content - unadsorbed\ protein\ content}{initial\ protein\ content} \times 100 \quad (4)$$

2.2.12. Foaming properties

The foaming properties were determined following the method described in [4]. The sample suspension (7 ml), at a concentration of 10 mg/ml, was homogenized at 10,000 rpm for 1 min. The initial foam volume (V_0) was measured immediately after homogenization, and again after 10 min (V_{10}) of storage. Foaming ability (FA) and foaming stability (FS) were calculated using the following equations:

$$FA\ (\%) = \frac{V_0 - 7}{7} \times 100 \quad (5)$$

$$FS\ (\%) = \frac{V_{10} - 7}{7} \times 100 \quad (6)$$

2.2.13. Statistical analysis

All values are presented as the mean ± standard deviation (SD) from the experiments conducted, at minimum, in triplicate. One-way analysis of variance was performed, and significant differences were analyzed by means of Duncan's test at $p < 0.05$, using the SPSS software (IBM SPSS statistics 20).

3. Results and discussion

3.1. Ultrasound effects on the structure of PBSA

3.1.1. Primary structure

SDS-PAGE was performed to visualize the distribution of molecular weights and changes in the primary structure. As illustrated in Fig. 1, the samples contained multiple subunits between 10 and 25 kDa. The electrophoresis patterns did not vary between samples, indicating that the ultrasound treatment did not alter the molecular weight of the protein. This suggests that the chemical bonds and primary structures were maintained as insufficient energy was supplied. Previous studies have also reported no change in the electrophoretic profile of sonicated *Moringa oleifera* seeds and scallop (*Patinopecten yessoensis*) proteins compared to the control [20,22].

3.1.2. Secondary structure

In general, the secondary structure of a protein can be quantitatively estimated by CD. Ultrasound decreased the content of α-helix and β-sheet structures, while increasing the content of the random coil (Table 1). The α-helix proportion decreased by 48% (20 min), 41% (40 min), and 33% (60 min), and the β-sheet content was reduced by 25% (20 min), 24% (40 min), and 24% (60 min), compared to the control.

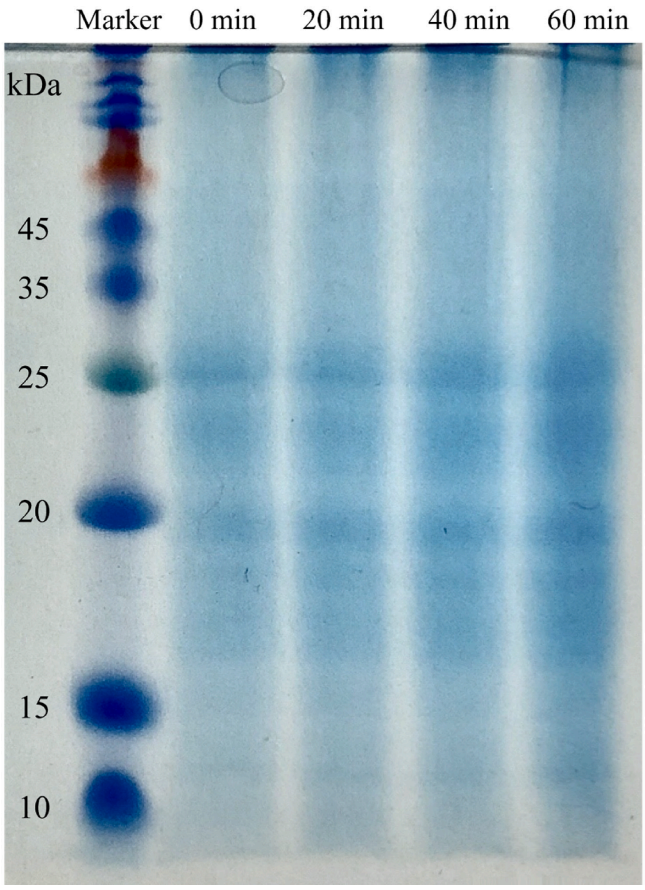

Fig. 1. Electrophoretic profile of proteins from black soybean Aquasoya with different ultrasonic time period (min).

Table 1
Secondary structure composition of proteins from black soybean Aquasoya in response to the time of ultrasound treatment.

Composition (%)	Ultrasonic time (min)			
	0	20	40	60
α-helix	19.35	10.13	11.40	12.90
β-sheet	45.02	33.78	34.24	34.01
β-turn	0	0	0	0
random coil	35.62	56.09	54.36	53.08

The random coil content increased drastically by 57% (20 min), 53% (40 min), and 49% (60 min). The interaction within a protein molecule determines its secondary structure. The alteration of the α-helix to a random coil structure corresponded to the partial unfolding of the protein caused by the cavitation effect of the ultrasound used in this process [9]. This observation can be attributed to the fact that the shear force, induced by cavitation, facilitates the collision of protein molecules, and weakens the intramolecular hydrogen bonding. Zou et al. [23], Tang et al. [22], and Cui et al. [24] also observed an increase in the unordered random coil content of chicken actomyosin and proteins from the *Moringa oleifera* and *Cyperus esculentus* seeds, respectively, over a certain range of ultrasonic power. Interestingly, as the ultrasonic time increased, the ratio of the α-helix and β-sheet also increased, implying that certain kinds of hydrogen bonding could be extended by excess treatment with ultrasound [25].

3.1.3. Tertiary structure

Intrinsic fluorescence is a widely used tool for detecting modifications in tertiary protein structures. The polarity of tryptophan/tyrosine

residues in the microenvironment is reflected in the changes in FI and maximum emission wavelength (λ_{max}) observed [23]. As illustrated in Fig. 2, after 20 min and 40 min of ultrasonication, λ_{max} increased from 342 nm to 345 nm and 348 nm, respectively, and decreased to 339 nm after 60 min of treatment. Furthermore, the FI was slightly decreased in the ultrasound-treated group compared to that observed in the control. The shift of λ_{max} to longer wavelengths (bathochromic shift) indicated that the previously buried tryptophan/tyrosine was exposed to a more polar environment. This partial unfolding corresponded to the alteration of the protein's tertiary structure provoked by strong shear stress, microsteaming, and turbulent flow by ultrasound. However, when the sonication time reached the upper limit, λ_{max} was blue-shifted, suggesting that excess ultrasonic time refolded the tertiary structure. This finding is supported by previous studies that observed a bathochromic shift of λ_{max} of walnut protein isolate treated after ultrasonic treatment [26].

3.2. H_0

ANS, a fluorescence probe, binds to hydrophobic sites on the protein molecules' surface, making it practical for monitoring the H_0 of proteins. H_0 is an efficient parameter that reflects the conformation and denaturation of proteins [9]. The effect of ultrasonic time on the PBSA is shown in Table 2. There were no significant differences in H_0 for any of the samples. This result conflicts with the observation reported in [27], wherein an increase in H_0 was observed for amaranth proteins that were treated with ultrasound at 100 W for 15–30 min. Furthermore, Cheng and Cui [28] also implemented ultrasonication on pea proteins and detected significant increments in H_0. Strong cavitation effects generated by ultrasound can decrease the intermolecular associations of protein molecules, resulting in the exposure of interior hydrophobic residues to the exterior surroundings [20]. In contrast, a study performed by Kang et al. [29] found that as the ultrasound power increased, the H_0 of chickpea protein increased to a certain level and decreased again. Excessive sonication might inordinately denature proteins, resulting in the protein aggregation and concealment of hydrophobic residues [23,24]. In the present study, ultrasound did not induce a change in H_0 at any time, which may be involved with the aforementioned mutual effect of ultrasound.

3.3. Particle size and distribution

Particle size and distribution are critical indicators of protein aggregation [30]. As shown in Table 2, the average particle size of the control was 633.8 ± 98.1 nm and was reduced by 10%, 35%, and 49% after 20, 40, and 60 min of ultrasonication, respectively. Furthermore, the application of ultrasonic treatment was followed by a decrease in PDI from 0.383 ± 0.040 to 0.340 ± 0.016, 0.283 ± 0.014, and 0.327 ± 0.011 with an increase in sonication time. The reduction in protein aggregate size and PDI after ultrasonication may be attributed to the mechanical vibration generated by the ultrasound, which facilitates the dissociation of larger protein aggregates into small particles, resulting in a narrow size distribution [19]. Li et al. [30] also obtained similar results; when proteins from sea cucumber gonads were subjected to ultrasound power intensities of 100 W and 200 W for 15 min, the proteins decreased in size and their distribution narrowed. However, several researchers have reported that increasing the rate of particle movement and collision, resulting from the cavitation effect, could lead to the formation of larger aggregates [14,31]. These conflicting results may be because protein types, the power of ultrasound, and the treatment time differed among the studies.

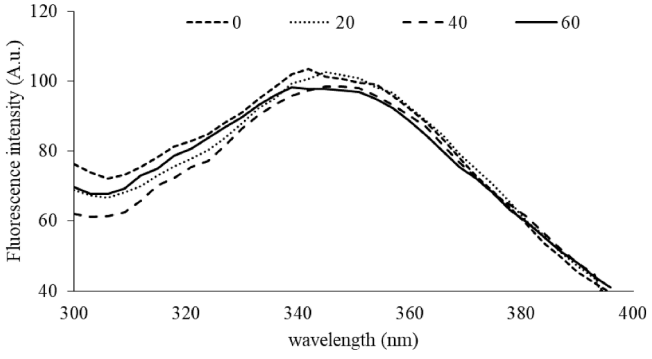

Fig. 2. Fluorescence spectra of proteins from black soybean Aquasoya with different ultrasonic time period (min).

Table 2
Effect of ultrasonic time on surface hydrophobicity, free sulfhydryl (SH) content, particle size, and polydispersity index of proteins from black soybean Aquasoya.

Ultrasonic time (min)	Surface hydrophobicity	Free SH content (μmol/g)	Particle size (nm)	Polydispersity index
0	3151 ± 276^{NS}	8.57 ± 0.00^{b}	633.8 ± 98.1^{a}	0.383 ± 0.040^{a}
20	3024 ± 603^{NS}	9.23 ± 0.02^{a}	573.2 ± 34.7^{a}	0.340 ± 0.016^{ab}
40	3098 ± 69^{NS}	9.38 ± 0.01^{a}	410.4 ± 17.5^{b}	0.283 ± 0.014^{c}
60	3138 ± 254^{NS}	9.07 ± 0.01^{ab}	324.6 ± 9.1^{b}	0.327 ± 0.011^{bc}

^{abc} Means within row with different letters indicate significant differences according to Duncan's test ($p < 0.05$).
^{NS}Not significant.

3.4. Free SH group content

The alteration of SH and disulfide bonds affects the conformational stability of protein molecules and, correspondingly, has a strong effect on their functionalities [32]. As shown in Table 2, the free SH content was significantly increased to 9.23, 9.38, and 9.07 μmol/g after 20, 40, and 60 min of ultrasound treatment, respectively, compared to the control (8.57 μmol/g). The reactive SH content was at its highest concentration after 40 min of ultrasound treatment; however, there was insignificant change in the SH content between the treated groups ($p > 0.05$). The disordered structure may be associated with an increase in SH content. Ultrasound can extend and unfold the protein structure, exposing the interior SH groups to the solvent [10,33]. This is in close agreement with the results of the structural characteristics mentioned in Section 3.1. The secondary and tertiary structures were altered, and the protein conformation became loose after ultrasound treatment. Additionally, Malik et al. [14] suggested that cavitational shear and pressure caused the breakage of disulfide bonds to form reactive SH groups, resulting in an increased free SH content. Kang et al. [29] observed that the SH content was time dependent. The free SH content of the chickpea protein isolate first increased and then decreased with increasing time of ultrasound treatment. The decrease in SH could be owing to the oxidation of the reactive SH by hydrogen peroxide in the cavities provoked during ultrasonication. In addition, the reduction in free SH content have resulted from the partial aggregation of proteins, which buried the SH groups inside again [30]. In this context, conformational alteration of proteins could be inferred through changes in the SH content.

3.5. Solubility

Solubility, an index of protein aggregation and denaturation, is not only one of the functionalities of the protein itself but also affects other functions such as emulsifying and foaming properties [22]. As shown in Fig. 3, ultrasound increased the solubility up to 0.980 g/g compared to the control (0.860 g/g), although differences between the treated samples was not significant. Solubility enhancement may be because of the

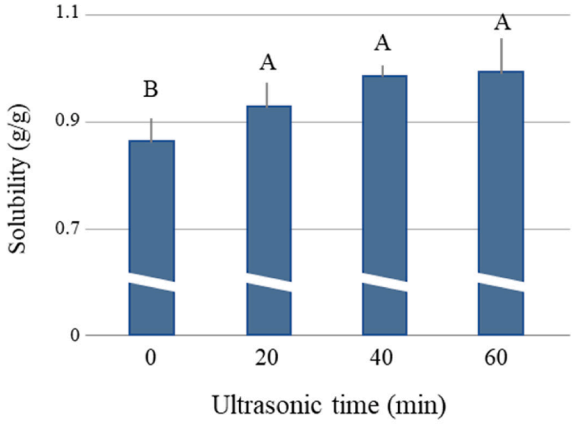

Fig. 3. Solubility of proteins from black soybean Aquasoya with different ultrasonic time period (min).

cavitation effect, which generates temperature rise, shear force, turbulent flow, micro-steaming, and shock waves [23]. This phenomenon is caused by the breakage of hydrogen bonds and hydrophobic interactions, which change the insoluble macromolecular substances into soluble protein aggregates [24]. This was in good agreement with the results in Section 3.3, where a decrease in the size of the protein aggregates was observed. Moreover, the increased surface area exerted by particle size reduction and exposure of hydrophilic groups by the partial unfolding of proteins enhanced the interaction between protein molecules and the surrounding water, improving the solubility. In agreement with this finding, Tang et al. [22] reported that water-soluble proteins from *Moringa oleifera* seeds showed increased solubility when treated with different amplitudes (20–100%) of ultrasound for 15 min. Martínez-Velasco et al. [13] also found that ultrasonication significantly improved the solubility of faba bean protein isolate.

3.6. Interfacial properties

Proteins, owing to their amphiphilicity, can adsorb at the colloidal interfaces, lower the interfacial tension, and thereby stabilize systems such as emulsions and foams [34]. Fig. 4 indicates that the ultrasound treatment positively affected the surface activity of PBSA. Regarding emulsification, ESI reached its maximum value at 37.61 min after 40 min of treatment, although EAI did not change in response to ultrasound (Fig. 4A). In addition, compared to the control, higher AP levels were detected in all the treated samples (Fig. 4B). In particular, the foam properties of PBSA were significantly improved by ultrasound ($p < 0.02$) (Fig. 4C). FC and FS were significantly enhanced with ultrasonic times of 20 min (47% and 163%, respectively), 40 min (55% and 181%, respectively), and 60 min (53% and 188%, respectively). The interfacial properties of proteins are significantly related to several factors such as particle size, molecular flexibility, and solubility [9,14,24,35]. Sha et al. [36] suggested that smaller particles have the capacity to better adsorb on to the oil-water or water-air interface. As discussed in Section 3.3, size-reduced particles were obtained using ultrasound treatment; thus, they could be loaded more onto the surface of the oil droplets. In addition, flexible protein molecules with disordered structures, attributed to partial denaturation, can be reorganized and reoriented at the interface [11]. Furthermore, the high solubility of proteins facilitates protein migration, adsorption, and rearrangement at the interface [34]. Overall, ultrasonication facilitates changes in the secondary and tertiary structures and encourages the dissolution of PBSA in the aqueous phase, thereby improving the interfacial properties. Taken together, this study reveals that the formation of a strong protein film can be achieved by modifying the structure and physicochemical properties of PBSA with ultrasonication.

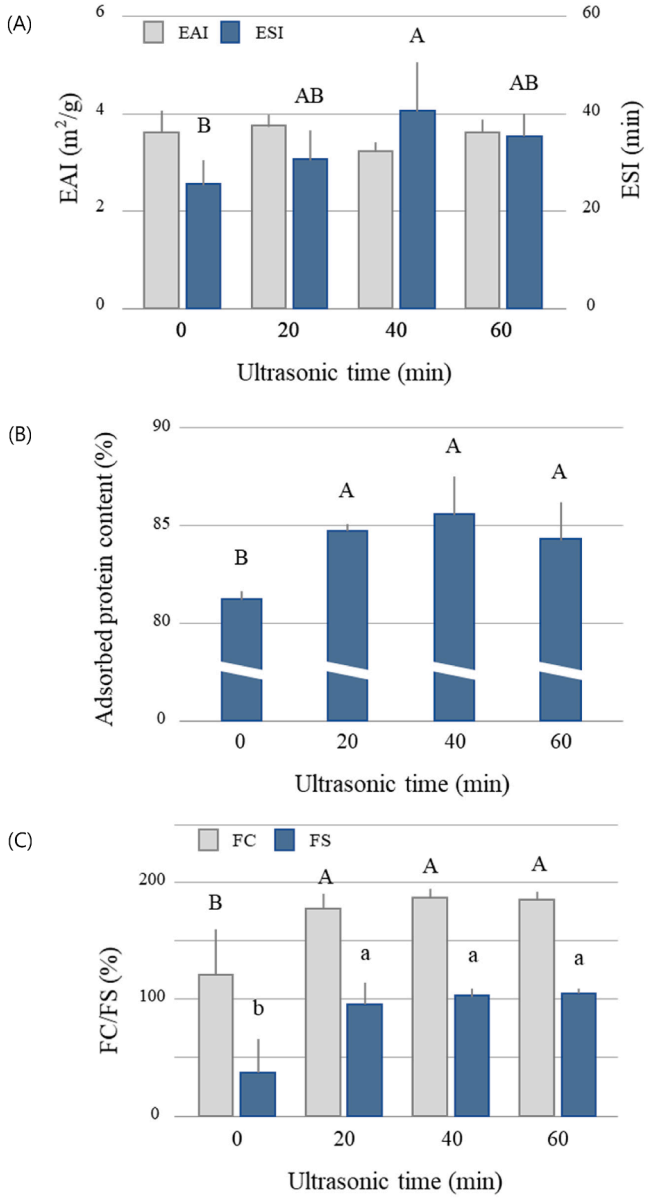

Fig. 4. Interfacial properties of proteins from black soybean Aquasoya with different ultrasonic time period (min). (A) emulsifying properties (emulsifying activity index (EAI) and emulsifying stability index (ESI)), (B) content of adsorbed protein on the surface of emulsions, and (C) foaming properties (foaming capacity (FC) and foaming stability (FS)).

4. Conclusion

This study demonstrates the potential of ultrasound as an efficient tool for modifying the structure and functional properties of PBSA. Ultrasound treatment loosened the protein structure, altering the ordered α-helix structure to a random coil and exposed non-polar groups to the exterior environment. Consequently, the SH groups buried inside were revealed, confirming the changes in the spatial conformation of the PBSA. Moreover, ultrasonication disrupted the intermolecular interactions, resulting in a decreased particle size and a more homogeneous distribution pattern. This modification imparted ultrasound-treated PBSA with improved powder solubility and interfacial properties such as good emulsion stability and foaming performance. It was observed that 40 min of sonication significantly improved the functionalities. Accordingly, the revalorization of black soybean Aquasoya, a by-product of soybean processing, can be achieved by ultrasonically

enhancing the functionalities of proteins recovered from the water. In conclusion, ultrasonicated PBSA is a promising food additive for manufacturing stable food products. Further studies should be conducted to apply PBSA to real food matrices, such as bakery and beverage products. This practical application can meet the needs of people who are unable to consume regular products owing to their dietary restrictions or preferences.

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CRediT authorship contribution statement

Min-Ji Kim: Methodology, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Weon-Sun Shin:** Supervision, Writing – review & editing, Project administration, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] E. Echeverria-Jaramillo, Y.-H. Kim, Y.-R. Nam, Y.-F. Zheng, J.Y. Cho, W.S. Hong, S. J. Kang, J.H. Kim, Y.Y. Shim, W.-S. Shin, Revalorization of the cooking water (aquafaba) from soybean varieties generated as a by-product of food manufacturing in Korea, Foods (Basel, Switzerland) 10 (2021) 2287, https://doi.org/10.3390/foods10102287.

[2] B. Kumari, B.K. Tiwari, M.B. Hossain, N.P. Brunton, D.K. Rai, Recent advances on application of ultrasound and pulsed electric field technologies in the extraction of bioactives from agro-industrial by-products, Food Bioprocess Technol. 11 (2017) 223–241, https://doi.org/10.1007/s11947-017-1961-9.

[3] J. Ampofo, M. Ngadi, Ultrasound-assisted processing: science, technology and challenges for the plant-based protein industry, Ultrason. Sonochem. 84 (2022) 105955, https://doi.org/10.1016/j.ultsonch.2022.105955.

[4] S. Kim, W.-S. Shin, Formation of a novel coating material containing lutein and zeaxanthin via a Maillard reaction between bovine serum albumin and fucoidan, Food Chem. 343 (2021) 128437, https://doi.org/10.1016/j.foodchem.2020.128437.

[5] Y.Y. Shim, Y. He, J.H. Kim, J.Y. Cho, V. Meda, W.S. Hong, W.-S. Shin, S.J. Kang, M. J.T. Reaney, Aquafaba from Korean soybean I: a functional vegan food additive, Foods (Basel, Switzerland) 10 (2021) 2433, https://doi.org/10.3390/foods10102433.

[6] Y.-H. Kim, W.-S. Shin, Evaluation of the physicochemical and functional properties of aquasoya (*Glycine max* Merr.) powder for vegan muffin preparation, Foods (Basel, Switzerland) 11 (2022) 591, https://doi.org/10.3390/foods11040591.

[7] W.-S. Shin, Aquasoya (Black soybean, *Glycine max* Merr.) powder as an egg replacer can give good functional properties for vegan mayonnaise, in: 2021 KoSFoST Int. Symp. Annu. Meet, 2021.

[8] W.-S. Shin, Aquasoya (Juinoonikong, *Rhynchosia nulubilis*) powder can give great functional properties for vegan meringue cookies, in: 2021 KoSFoST Int. Symp. Annu, 2021.

[9] S. Yan, J. Xu, S. Zhang, Y. Li, Effects of flexibility and surface hydrophobicity on emulsifying properties: ultrasound-treated soybean protein isolate, LWT. 142 (2021) 110881, https://doi.org/10.1016/j.lwt.2021.110881.

[10] H. Cao, R. Sun, J. Shi, M. Li, X. Guan, J. Liu, K. Huang, Y. Zhang, Effect of ultrasonic on the structure and quality characteristics of quinoa protein oxidation aggregates, Ultrason. Sonochem. 77 (2021) 105685, https://doi.org/10.1016/j.ultsonch.2021.105685.

[11] J.A. Resendiz-Vazquez, J.A. Ulloa, J.E. Urías-Silvas, P.U. Bautista-Rosales, J. C. Ramírez-Ramírez, P. Rosas-Ulloa, L. González-Torres, Effect of high-intensity ultrasound on the technofunctional properties and structure of jackfruit (*Artocarpus heterophyllus*) seed protein isolate, Ultrason. Sonochem. 37 (2017) 436–444, https://doi.org/10.1016/j.ultsonch.2017.01.042.

[12] G. Karabulut, O. Yemiş, Modification of hemp seed protein isolate (*Cannabis sativa* L.) by high-intensity ultrasound treatment. Part 1: functional properties, Food Chem. 375 (2022) 131843, https://doi.org/10.1016/j.foodchem.2021.131843.

[13] A. Martínez-Velasco, C. Lobato-Calleros, B.E. Hernández-Rodríguez, A. Román-Guerrero, J. Alvarez-Ramirez, E.J. Vernon-Carter, High intensity ultrasound treatment of faba bean (*Vicia faba* L.) protein: effect on surface properties, foaming ability and structural changes, Ultrason. Sonochem. 44 (2018) 97–105, https://doi.org/10.1016/j.ultsonch.2018.02.007.

[14] M.A. Malik, H.K. Sharma, C.S. Saini, High intensity ultrasound treatment of protein isolate extracted from dephenolized sunflower meal: effect on physicochemical and functional properties, Ultrason. Sonochem. 39 (2017) 511–519, https://doi.org/10.1016/j.ultsonch.2017.05.026.

[15] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature. 227 (1970) 680–685, https://doi.org/10.1038/227680a0.

[16] H.-W. Park, D.-Y. Kim, W.-S. Shin, Fucoidan improves the structural integrity and the molecular stability of β-lactoglobulin, Food Sci. Biotechnol. 27 (2018) 1247–1255, https://doi.org/10.1007/s10068-018-0375-4.

[17] B. Ahmad, S. Parveen, R.H. Khan, Effect of albumin conformation on the binding of ciprofloxacin to human serum albumin: a novel approach directly assigning binding site, Biomacromolecules 7 (2006) 1350–1356, https://doi.org/10.1021/bm050996b.

[18] A. Kato, S. Nakai, Hydrophobicity determined by a fluorescence probe method and its correlation with surface properties of proteins, Biochim. Biophys. Acta - Protein Struct. 624 (1980) 13–20, https://doi.org/10.1016/0005-2795(80)90220-2.

[19] R. Li, Y.L. Xiong, Ultrasound-induced structural modification and thermal properties of oat protein, LWT. 149 (2021) 111861, https://doi.org/10.1016/j.lwt.2021.111861.

[20] Q. Ding, G. Tian, X. Wang, W. Deng, K. Mao, Y. Sang, Effect of ultrasonic treatment on the structure and functional properties of mantle proteins from scallops (*Patinopecten yessoensis*), Ultrason. Sonochem. 79 (2021) 105770, https://doi.org/10.1016/j.ultsonch.2021.105770.

[21] S.-J. Jeong, S. Kim, E. Echeverria-Jaramillo, W.-S. Shin, Effect of the emulsifier type on the physicochemical stability and in vitro digestibility of a lutein/zeaxanthin-enriched emulsion, Food Sci. Biotechnol. 30 (2021) 1509–1518, https://doi.org/10.1007/s10068-021-00987-9.

[22] S.-Q. Tang, Q.-H. Du, Z. Fu, Ultrasonic treatment on physicochemical properties of water-soluble protein from *Moringa oleifera* seed, Ultrason. Sonochem. 71 (2021) 105357, https://doi.org/10.1016/j.ultsonch.2020.105357.

[23] Y. Zou, P. Xu, H. Wu, M. Zhang, Z. Sun, C. Sun, D. Wang, J. Cao, W. Xu, Effects of different ultrasound power on physicochemical property and functional performance of chicken actomyosin, Int. J. Biol. Macromol. 113 (2018) 640–647, https://doi.org/10.1016/j.ijbiomac.2018.02.039.

[24] Q. Cui, L. Wang, G. Wang, A. Zhang, X. Wang, L. Jiang, Ultrasonication effects on physicochemical and emulsifying properties of *Cyperus esculentus* seed (tiger nut) proteins, LWT. 142 (2021) 110979, https://doi.org/10.1016/j.lwt.2021.110979.

[25] Y. Meng, Z. Liang, C. Zhang, S. Hao, H. Han, P. Du, A. Li, H. Shao, C. Li, L. Liu, Ultrasonic modification of whey protein isolate: implications for the structural and functional properties, LWT. 152 (2021) 112272, https://doi.org/10.1016/j.lwt.2021.112272.

[26] F. Zhao, X. Zhai, X. Liu, M. Lian, G. Liang, J. Cui, H. Dong, W. Wang, Effects of high-intensity ultrasound pretreatment on structure, properties, and enzymolysis of walnut protein isolate, Molecules. 27 (2021) 208, https://doi.org/10.3390/molecules27010208.

[27] A.B. Tomé Constantino, E.E. Garcia-Rojas, Modifications of physicochemical and functional properties of amaranth protein isolate (*Amaranthus cruentus* BRS Alegria) treated with high-intensity ultrasound, J. Cereal Sci. 95 (2020) 103076, https://doi.org/10.1016/j.jcs.2020.103076.

[28] J. Cheng, L. Cui, Effects of high-intensity ultrasound on the structural, optical, mechanical and physicochemical properties of pea protein isolate-based edible film, Ultrason. Sonochem. 80 (2021) 105809, https://doi.org/10.1016/j.ultsonch.2021.105809.

[29] S. Kang, J. Zhang, X. Guo, Y. Lei, M. Yang, Effects of ultrasonic treatment on the structure, functional properties of chickpea protein isolate and its digestibility in vitro, Foods (Basel, Switzerland) 11 (2022) 880, https://doi.org/10.3390/foods11060880.

[30] H. Li, Y. Hu, X. Zhao, W. Wan, X. Du, B. Kong, X. Xia, Effects of different ultrasound powers on the structure and stability of protein from sea cucumber gonad, LWT. 137 (2021) 110403, https://doi.org/10.1016/j.lwt.2020.110403.

[31] Q. Liang, X. Ren, W. Qu, X. Zhang, Y. Cheng, H. Ma, The impact of ultrasound duration on the structure of β-lactoglobulin, J. Food Eng. 292 (2021) 110365, https://doi.org/10.1016/j.jfoodeng.2020.110365.

[32] J.K.P. Weder, H.-D. Belitz, Protein | functional properties, Encycl. Food Sci. Nutr. (2003) 4835–4841, https://doi.org/10.1016/b0-12-227055-x/00982-2.

[33] M.M. Rahman, B.P. Lamsal, Ultrasound-assisted extraction and modification of plant-based proteins: impact on physicochemical, functional, and nutritional properties, Compr. Rev. Food Sci. Food Saf. 20 (2021) 1457–1480, https://doi.org/10.1111/1541-4337.12709.

[34] H. Hu, I.W.Y. Cheung, S. Pan, E.C.Y. Li-Chan, Effect of high intensity ultrasound on physicochemical and functional properties of aggregated soybean β-conglycinin

and glycinin, Food Hydrocoll. 45 (2015) 102–110, https://doi.org/10.1016/j.foodhyd.2014.11.004.
[35] W. Ma, J. Wang, X. Xu, L. Qin, C. Wu, M. Du, Ultrasound treatment improved the physicochemical characteristics of cod protein and enhanced the stability of oil-in-water emulsion, Food Res. Int. 121 (2019) 247–256, https://doi.org/10.1016/j.foodres.2019.03.024.
[36] L. Sha, A.O. Koosis, Q. Wang, A.D. True, Y.L. Xiong, Interfacial dilatational and emulsifying properties of ultrasound-treated pea protein, Food Chem. 350 (2021) 129271, https://doi.org/10.1016/j.foodchem.2021.129271.