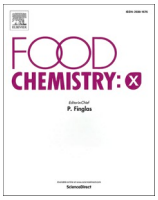
Food Chemistry: X 21 (2024) 101151

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Mechanism of ultrasonic enhancement of the gelling properties of salted ovalbumin-cooked soybean isolate hybrid gels

Ji'en Tan [a,b,c,1], Wei Qiu [d,1], Na Wu [a,b], Lilan Xu [a,b], Shuping Chen [a,b], Yao Yao [a,b], Mingsheng Xu [a,b], Yan Zhao [a,b,*], Yonggang Tu [a,b,*]

[a] Jiangxi Key Laboratory of Natural Products and Functional Food, Jiangxi Agricultural University, Nanchang 330045, China
[b] Agricultural Products Processing and Quality Control Engineering Laboratory of Jiangxi, Jiangxi Agricultural University, Nanchang 330045, China
[c] College of Animal Science and Technology, Jiangxi Agricultural University, Nanchang 330045, China
[d] Engineering Research Center of Biomass Conversion, Ministry of Education, Nanchang University, Nanchang 330047, China

ARTICLE INFO

Keywords:
Ovalbumin
Ultrasound treatment
Salted egg white
Hybrid gel
Gelling properties

ABSTRACT

The influence of ultrasonic processing on the physicochemical characteristics, microstructure, and intermolecular forces of the hybrid gels obtained by heating the mixtures of different ratios of salted ovalbumin (SOVA)-cooked soybean protein isolate (CSPI) was investigated. With the growth of SOVA addition, ζ-potential in absolute value, cohesiveness, water-holding capacity (WHC), surface hydrophobicity, and the content of soluble protein of the hybrid gels decreased ($P < 0.05$), while the hardness, T_2 relaxation time of the hybrid gels increased ($P < 0.05$). And the compactness of the network structure of the hybrid gel increased with the increase of SOVA addition. After being treated with ultrasound, significant increases ($P < 0.05$) of ζ-potential in absolute value, cohesiveness, WHC, and surface hydrophobicity of the hybrid gels were observed. In general, ultrasonic processing is one of the effective means to improve the gel properties of SOVA-CSPI hybrid gels.

1. Introduction

Salted egg yolk, with a tender taste and rich in vermillion oil, was popular with consumers and was often used to make dishes, pastries, and egg yolk-flavored foods (Xu et al., 2018). However, there were over 10,000 tons of salted egg whites produced each year as the by-products of the production of salted egg yolk in China (Du et al., 2022). Due to the high content of salt (about 4 %-7%), the salted egg whites were often discarded directly, resulting in environmental pollution and the tremendous wastage of high-protein resources (Ding, Li, & Cao, 2014). Therefore, it is of great significance for both the protection of the environment and the industrial economic development to resourcefully utilize the salted egg white. Making egg tofu from salted egg whites was one of the means to realize the resourceful utilization of salted egg whites by using the gelling property of the protein.

Soybean protein isolate (SPI), the main component in soybeans, was usually used as a primary ingredient for making tofu (Taha et al., 2018). However, the solubility of the SPI was poor, and heat treatment was an effective physical method to improve the poor solubility of SPI (Wen et al., 2023). After heating treatment, not only the solubility but also the other functional properties (such as gelling) of SPI improved due to the unfolding of the protein structures and the exposure of the buried hydrophobic groups (Wen et al., 2023). In the preliminary experiments, we found that the tofu made by directly heating the mixture of salted egg whites and cooked soybean protein isolate (CSPI) was fragile. There were researchers reported that a firmer texture of the tofu produced by high-intensity ultrasound was formed compared with that of the tofu produced by traditional methods (Lin, Lu, Hsieh, & Kuo, 2016). This showed that ultrasonic treatment was one of the effective means to improve the gel properties of tofu. Ultrasound, as one of the physical means to improve the functional properties of proteins, has been widely used in the food industry (Lu et al., 2022). The functional properties of the proteins were significantly affected by ultrasound due to the modification of proteins and the changes in the protein conformation caused by the cavitation effect generated by ultrasound (Higuera-Barraza, Del Toro-Sanchez, Ruiz-Cruz, & Marquez-Rios, 2016; Hu, Cheung, Pan, & Li-Chan, 2015).

Therefore, ultrasonic treatment might be an effective means to

* Corresponding authors at: Jiangxi Key Laboratory of Natural Products and Functional Food, Jiangxi Agricultural University, Nanchang 330045, China.
E-mail addresses: zhaoyan@jxau.edu.cn (Y. Zhao), tygzy1212@jxau.edu.cn (Y. Tu).
[1] These authors are co-first authors of the article.

https://doi.org/10.1016/j.fochx.2024.101151
Received 23 November 2023; Received in revised form 11 January 2024; Accepted 17 January 2024
Available online 20 January 2024
2590-1575/© 2024 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

improve the gel properties of tofu made by the mixtures of salted egg white-CSPI. At the same time, it was of great meaningful to elucidate the mechanism of ultrasonic treatment to enhance the gel properties of salted egg white-CSPI hybrid gel. However, due to the complex composition of proteins in egg white, the mechanism of ultrasonic treatment to enhance the gel properties of salted egg white-CSPI hybrid gel was difficult to elucidate directly. Ovalbumin (OVA), the main protein in egg white and accounted for 54 %–65 % of the total egg white protein content, was selected as a simplified alternative to elucidate the mechanism of ultrasonic treatment to enhance the gel properties of salted egg white-CSPI hybrid gel (Xue, Xu, et al., 2021). In this study, the hybrid gels were prepared by heating the mixtures (without or with ultrasonic treatment) consisting of different ratios of salted ovalbumin (SOVA)-CSPI solutions. The variations in the physicochemical characteristics, microstructure, and intermolecular interactions of hybrid gels were evaluated to get insight into the mechanism of ultrasonic treatment to enhance the gel properties of the hybrid gel. This study provided a theoretical basis for the ultrasound-assisted preparation of salted egg whites into tofu, which was conducive to improving the utilization of salted egg whites and reducing resource waste.

2. Materials and methods

2.1. Materials

The companies of Sigma Chemical Co. (St. Louis, MO, USA) and Yuwang Food Ltd. (Shandong, China) provided the OVA and SPI, respectively. And salt was purchased from Fuda Salt Chemical Co., Ltd. All other chemicals at analytical grade were employed in this study.

2.2. Obtaining SOVA-CSPI hybrid solution and gels

The preparation of SOVA-CSPI hybrid solution: the SOVA solution was prepared as follows: before being stirred for 30 min employing a magnetic stirrer, the OVA powder was blended with the distilled water at a ratio of 1:10 (w/w), followed by the centrifugation for 15 min at 17,530×g. And 5 % salt (w/w) was added into the supernatant, followed by magnetic stirring for 5 min to dissolve. The CSPI solution was prepared as follows: the SPI powder was mixed with distilled water and then stirred for 4 h to obtain SPI solution (10 %, w/w), followed by heating for 10 min in boiling water to obtain CSPI. After being cooled to room temperature, the SOVA solution was mixed with the CSPI solution at a ratio of 1:4, 1:3, 3:7, 2:3, and 1:1 (w/w), respectively. Then the mixed solution was homogenized for 2 min at 10,000 rpm by using a T25 homogenizer (IKA Instruments Ltd., Staufen, Germany) to obtain a hybrid solution.

The preparation of SOVA-CSPI hybrid gel: after being stood for 3 h at 4 °C, the hybrid solutions (40 g) were treated with different power of ultrasound (0, 300 W) in an ice bath for 15 min by employing an ultrasound processor (JY92-IIN, Ningbo Xinzhi Biotechnology Co., Zhejiang, China). The pulse mode applied in this study was 3 s ON and 3 s OFF. After ultrasonic treatment, the collagen casings with a diameter of 14 mm were used to pack the hybrid solutions, then heated for 30 min at 90 °C, followed by being cooled rapidly using ice water. Finally, the maturation of the samples was conducted by placing them at 4 °C overnight.

2.3. Determination of ζ-potential

Similar to the method described by Yang, Gao, and Yang, (2020), the ζ-potential of the SOVA-CSPI hybrid gels was determined. Briefly, 2 g of hybrid gels was blended with 18 mL distilled water and then homogenized for 1 min at 10,000 rpm. After the homogenization, the mixtures were centrifuged for 20 min at 17,530×g. After being diluted 10 times, the ζ-potential of the supernatant was assayed by employing a Zetasizer Nano ZEN2600 instrument (Malvern Instruments Ltd., Malvern, UK).

2.4. Texture profile analysis (TPA)

The hardness and cohesiveness of SOVA-CSPI hybrid gels were determined according to the method described by Sow, Toh, Wong, and Yang, (2019) with some modifications, employing a TA.XT. Plus texture analyzer (Stable Micro Systems Ltd., UK), which was loaded with a cylindrical probe (P/36R). Before the determination, cylinders with a diameter of 14 mm and a height of 10 mm were obtained by cutting the hybrid gels. The test conditions in this study were as follows: trigger force 5 g, compression ratio 40 %, test speed 2 mm/s, pre-test and post-test speed 5 mm/s.

2.5. Determination of water-holding capacity (WHC)

The WHC of the SOVA-CSPI hybrid gels was assayed regarding the method described by Xue, Tu, et al. (2021). Before being centrifuged at 17,530×g for 30 min, the weighed samples were put into ultra-filtration centrifuge tubes. The centrifuged samples were then weighed. The WHC of the hybrid gels was calculated as follows:

$$\mathrm{WHC}\ (\%) = W_2/W_1 \times 100 \quad (1)$$

Where W_1 and W_2 represented the weight of the hybrid gels before and after centrifugation, respectively.

2.6. Determination of microstructure

According to the method described by Li et al. (2022), a scanning electron microscope (SU8100, Tokyo Hitachi Co., Ltd., Tokyo, Japan) was employed to observe the microstructure of the SOVA-CSPI hybrid gels. Small cuboids ($3 \times 3 \times 1\ mm^3$), which were obtained by cutting the hybrid gels, were fixed with 2.5 % glutaraldehyde for 12 h, followed by washing with phosphate buffer (0.4 M) 3 times and dehydrating with ethanol. Before freeze-drying, those small cuboids were frozen completely by placing them at −80 °C. The microstructure of the samples after being platted with gold was observed by scanning electron microscope in low vacuum mode. And the observation magnification was 5,000×.

2.7. Determination of low-field nuclear magnetic resonance (LF-NMR)

The relaxation time (T_2) of the SOVA-CSPI hybrid gels was assayed according to the method described by Shao et al. (2016), employing a Niumag low-field pulsed NMR analyzer (Niumag Co., Ltd., Shanghai, China). Before loading into the nuclear magnetic tube, the hybrid gels were smashed, followed by transferring to the magnetic field center of the NRM analyzer. The measurement mode of the Carr–Purcell–Meiboom–Gill (CPMG) sequence was applied to measure the T_2 of the gel sample. Before the test, the repetition time, number of scan repetitions, number of echoes, and NECH were set as 1000.000 ms, 4, 140, and 4,000, respectively.

2.8. Determination of surface hydrophobicity

Similar to the method of Chang et al. (2016), a Fluorescence Spectrophotometer (F-7000, Hitachi Co., Ltd., Tokyo, Japan) was employed to assay the surface hydrophobicity of the SOVA-CSPI hybrid gels. The sample (2 g) was homogenized with 18 mL phosphate buffer solution, followed by centrifuging for 20 min at 17,530×g. Before adding 200 μL ANS (0.69 mM), a final protein concentration of 0.3 mg/mL dilution was obtained by diluting the supernatant. After being placed at room temperature for 10 min in the dark, the dilutions were scanned at an excitation wavelength of 380 nm, an emission wavelength of 400–600 nm, and a slit width of 5 nm.

2.9. Determination of soluble protein content

The SOVA-CSPI hybrid gels (2 g) were blended with phosphate buffer solution (18 mL), followed by homogenization and centrifugation at 17,530×g for 20 min. Then the BCA Protein Quantitative Assay kit was employed to obtain the protein concentration of the supernatant.

2.10. Selective protein solubility

Referring to the method of Tan et al. (2022), the selective protein solubility of SOVA-CSPI hybrid gels was determined. Four different solvents were used for the assay: S1 (0.6 M NaCl), S2 (0.6 M NaCl + 1.5 M urea), S3 (0.6 M NaCl + 8 M urea), and S4 (0.6 M NaCl + 8 M urea + 0.5 M β-mercaptoethanol). One gram of hybrid gels was homogenized with 9 mL S1 at 12,000 rpm for 2 min, then centrifuged at 17,530×g for 20 min, followed by the separation of precipitate and supernatant. The precipitate was treated in 9 mL of S2 in the same way as in S1. The same treatment was also conducted sequentially in S3 and S4. The BCA method was employed to obtain the protein concentration in the four supernatants. Since β-mercaptoethanol would interfere with the determination of protein, the supernatant separated from S4 needed to be dialyzed by S1 for 24 h to remove β-mercaptoethanol. The results were presented as a percentage of the protein concentration in each solvent with respect to the sum of protein concentrations in the four solvents.

3. Results and discussion

3.1. Variations of the ζ-potential in SOVA-CSPI hybrid gels

Since the charge on the surface of protein can be characterized by the ζ-potential, the ζ-potential can be used to indicate the stabilization and aggregation of protein gels (Xue, Liub, Wu, Zhang, Tua, & Zhao, 2022). The influence of ultrasonic treatment on the ζ-potential of SOVA-CSPI hybrid gels was displayed in Fig. 1A. As shown, all the ζ-potential values were negative and the absolute values were greater than 30 mV, indicating the relatively stable of the SOVA-CSPI hybrid gel system. Theζ-potential in the absolute value of SOVA-CSPI hybrid gels untreated or treated with ultrasound all progressively declined ($P < 0.05$) with the growth of SOVA addition, from 37.6 mV (0 W) and 39.4 mV (300 W) to

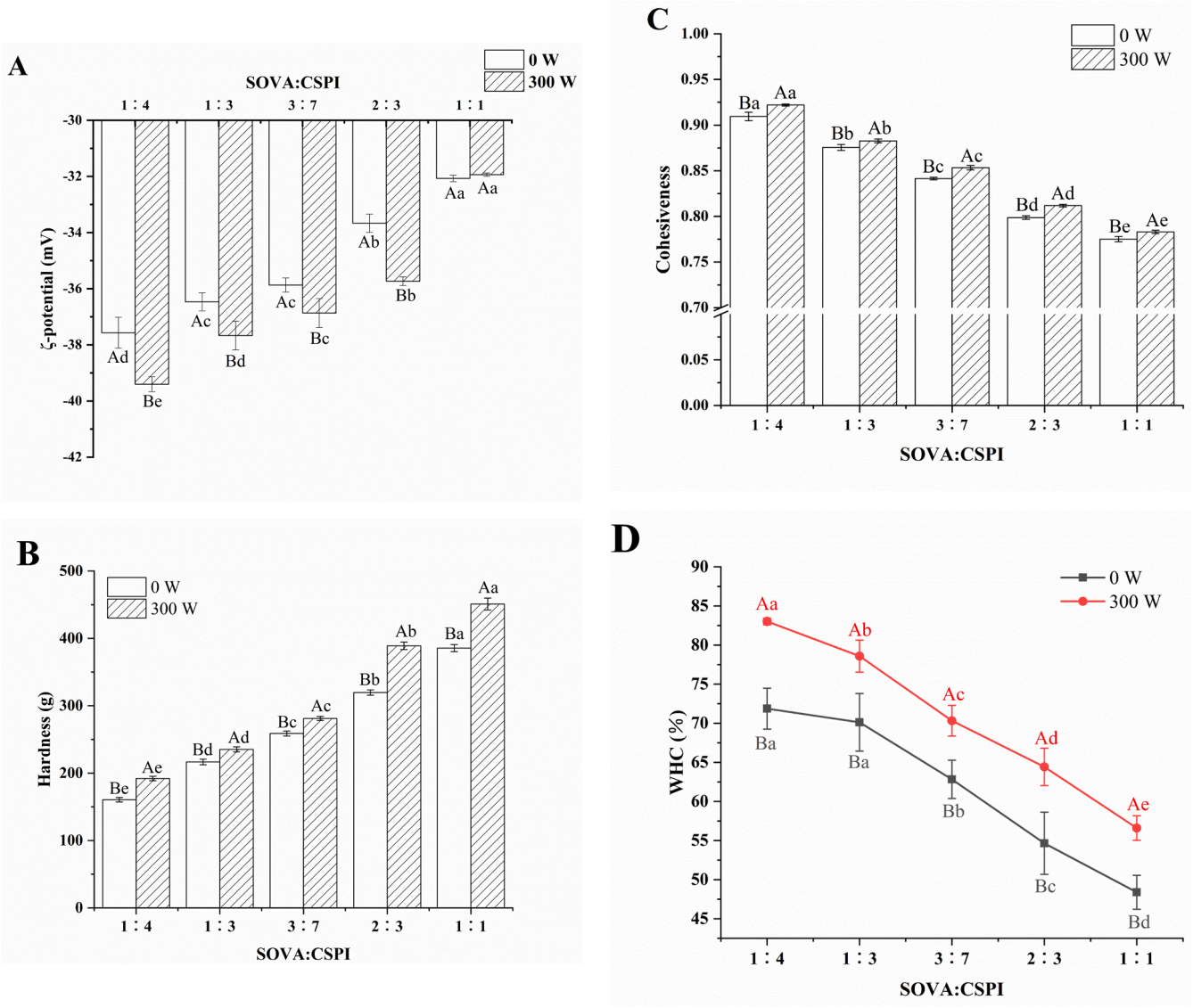


Fig. 1. Changes in the ζ-potential (A), hardness (B) and cohesiveness (C), and WHC (D) of different ratios of SOVA-CSPI hybrid gels untreated or treated with ultrasound. Different uppercase letters (A-B) indicated significant differences between samples untreated or treated with ultrasound ($P < 0.05$), and different lowercase letters (a-e) indicated significant differences between samples of different ratios ($P < 0.05$).

32.1 mV (0 W) and 31.9 mV (300 W), respectively. This might be due to the increase of Na^+ in the hybrid gel caused by the increase of SOVA addition, which decreased the relativistic thickness of the protein double electric layer. The electrostatic repulsion formed between the molecules of protein can be reflected by the ζ-potential. The larger the absolute value of the ζ-potential, the stronger the electrostatic repulsion, and vice versa (Han et al., 2022; Yu et al., 2020). Xue, Tu, et al. (2021) found that when the absolute value of ζ-potential decreased, the stability of the protein dispersions decreased or tended to aggregate. The results of ζ-potential demonstrated the weaker electrostatic repulsion in the SOVA-CSPI hybrid gels between protein molecules with the incremental addition of SOVA, leading to the agglomeration of protein molecules or the decrease in the stability of the hybrid gel system. A remarkable increase ($P < 0.05$) in the absolute value of ζ-potential was observed after ultrasonic treatment, which suggested that the protein structure of SOVA-CSPI hybrid gel was partially unfolded and partially cross-linked due to ultrasonic treatment, enhancing the stability of the hybrid gels (Xin et al., 2021).

3.2. Variations of the textural properties in SOVA-CSPI hybrid gels

As illustrated in Fig. 1B, the stiffness of the SOVA-CSPI hybrid gels dramatically increased ($P < 0.05$) with the increase of SOVA addition. With the growth of SOVA addition (from ratio 1:4 to ratio 1:1), the stiffness of the hybrid gels untreated or treated with ultrasound increased by about 136 % (0 W) and 135 % (300 W), respectively. The content of salt in the SOVA-CSPI hybrid system increased with the increase of SOVA addition, thus reducing the repulsive force between protein molecules, which was demonstrated by the previous results of the ζ-potential (Fig. 1A). The shielding effect of metal cations on the negative charge of proteins decreased the intermolecular repulsion of proteins, leading to the enhancement of electrostatic interactions, thus promoting the aggregation of proteins (Xu et al., 2017). The enhancement in the aggregation of proteins was favorable to enhance the stiffness of the hybrid gels. Compared with the stiffness of the hybrid gels untreated with ultrasound, the stiffness of the hybrid gels treated with ultrasound at 300 W exhibited an increase ($P < 0.05$). This might be ascribed to the fact that the cavitation effect generated by ultrasonic treatment partially unfolded the SOVA and CSPI protein structures, and some groups were partially cross-linked, thus improving the stiffness of the hybrid gels after being treated with ultrasound (Xin et al., 2021).

Cohesiveness can reflect the strength of the internal binding ability of the gel. Higher cohesiveness indicated that the gel was more resistant to external compression and maintained an intact network structure (Han et al., 2022; Yu et al., 2020). As displayed in Fig. 1C, the cohesiveness of the SOVA-CSPI hybrid gels treated or untreated with ultrasound declined ($P < 0.05$) with the growth of SOVA addition. There were two possible reasons, one of which was that with the increase of SOVA addition, the electrostatic repulsion formed between the molecules of protein was weakened (as discussed in Section 3.1), which promoted the gathering of protein molecules in the hybrid gels and hindered the unfolding of protein molecules, thus causing the weakening of cohesiveness. Another reason was that thermal denaturation led to the separation of SOVA and CSPI during gelation, and SOVA preferentially aggregated into agglomerates, reducing cross-linking and thus reducing cohesiveness. And the cohesiveness of ultrasonically treated hybrid gels was higher ($P < 0.05$) than that of non-ultrasonically treated hybrid gels. This result further suggested that the treatment of ultrasound might have partially unfolded the protein molecular structure in SOVA-CSPI hybrid gels, and some groups were partially cross-linked, enhancing the resistance of the hybrid gels to compression (Xin et al., 2021).

3.3. Variations of the WHC in SOVA-CSPI hybrid gels

WHC can reflect the extent of the interaction between protein and water in the gel. As shown in Fig. 1D, with the increased SOVA addition, the WHC of the SOVA-CSPI hybrid gels without or with the treatment of ultrasound all decreased ($P < 0.05$). In the gel system, the changes in WHC were related to the interactions of protein-water and protein–protein (Lan et al., 2019). And the electrostatic repulsion formed between the molecules of protein had an effect on the amount of protein-water binding (Wagner, Sorgentini, & Anon, 2000). Combined with the findings in terms of ζ-potential (Fig. 1A), the electrostatic repulsion formed between the molecules of protein gradually decreased with the growth of SOVA addition, which promoted the gathering of protein molecules in the hybrid gel and hindered the unfolding of protein molecules, causing the reduction of protein-water binding, thus resulting in the decrease of WHC. Other researchers have reported similar results that the WHC of the egg white gel decreased with the NaCl addition (Li, Li et al., 2018).

After the treatment of ultrasound, the WHC of the SOVA-CSPI hybrid gels increased, which might be due to the cavitation effect generated by ultrasonic treatment caused the folded protein structure to unfold, thus forming a more stable gel structure (Xue, Tu, et al., 2021). This result was identical to the results of ζ-potential (Fig. 1A), stiffness (Fig. 1B) and cohesiveness (Fig. 1C). And the treatment of ultrasound strengthened the interaction between protein and water molecules, making water molecules tightly wrapped in the protein network structure and less likely to be thrown out, thus improving the WHC of SOVA-CSPI hybrid gels.

3.4. Variations of the microstructure in SOVA-CSPI hybrid gels

The microstructure, which was also an important indicator to characterize the gel properties, can reflect the homogeneity of the gel network structure and the aggregation of protein molecules (Sow, Chong, Liao, & Yang, 2018). As shown in Fig. 2, all of the SOVA-CSPI hybrid gels showed a compact network structure. Some researchers have reported that the denaturation temperature of SPI was 92.5 °C, and SPI would form a more porous network structure after being modified at a higher temperature (Tan, Ying-Yuan, & Gan, 2014; Wu et al., 2020). The denaturation temperature of OVA (72 °C) was lower than that of SPI, so the gelation process of OVA was faster than SPI (Niu et al., 2018). Due to the interaction between OVA and CSPI, the microscopic pores of the CSPI gel can be filled by the random aggregates that formed the structure of OVA gel, leading to the hybrid gels exhibiting a compact network structure. Other researchers also observed similar results that a more dense structure was observed after the microscopic pores in the SPI gel were filled by hen egg proteins (Zhang et al., 2019). With the growth of SOVA addition, the network structure of the hybrid gels without or with the treatment of ultrasound all became denser and the pores were smaller, which might be attributed to the increase in the extent of protein aggregation. However, with the growth of SOVA addition, the surface structure of the hybrid gels without ultrasonic treatment became rough. With the increase of SOVA addition, the size of the aggregates formed by SOVA proteins aggregating with each other was larger than the gaps in the CSPI network structure, resulting in partial embedding and partial aggregation of SOVA proteins on the surface of the network structure of CSPI gel.

In addition, a more uniform and denser microstructure of the hybrid gels was observed after being treated with ultrasound. One of the reasons for this observation might be that the ultrasonic cavitation effect lowered the particle size of SOVA proteins, resulting in a more uniform dispersion of SOVA proteins in the CSPI network structure (Hu et al., 2013). Another possible reason was that ultrasonic treatment can unfold the tertiary structure of proteins, exposing some functional groups (e.g., hydrophobic groups) that rapidly interact with each other, thus promoting agglomeration of proteins and the development of hybrid gels with denser structure (Renkema & van Vliet, 2002).

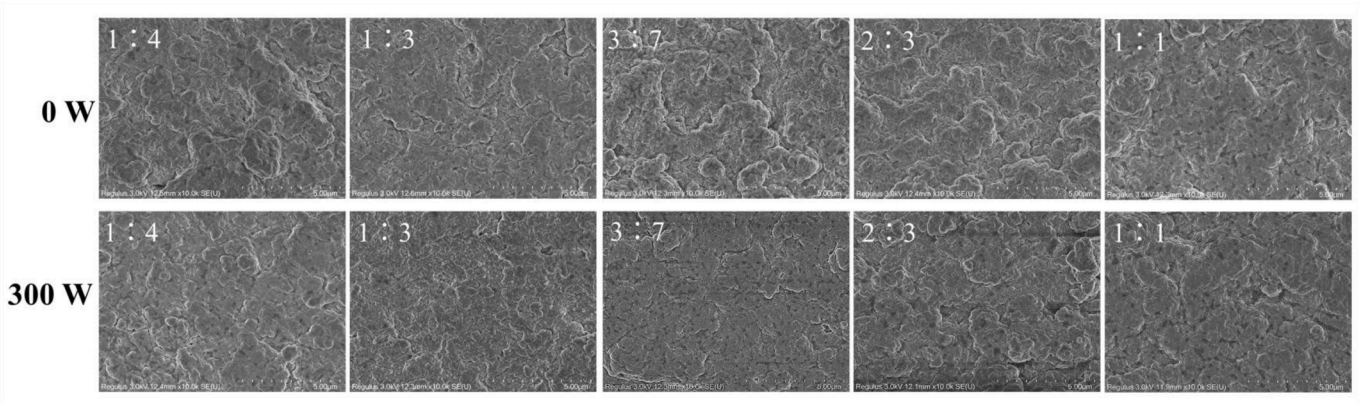


Fig. 2. Changes in the microstructure of different ratios of SOVA-CSPI hybrid gels untreated or treated with ultrasound.

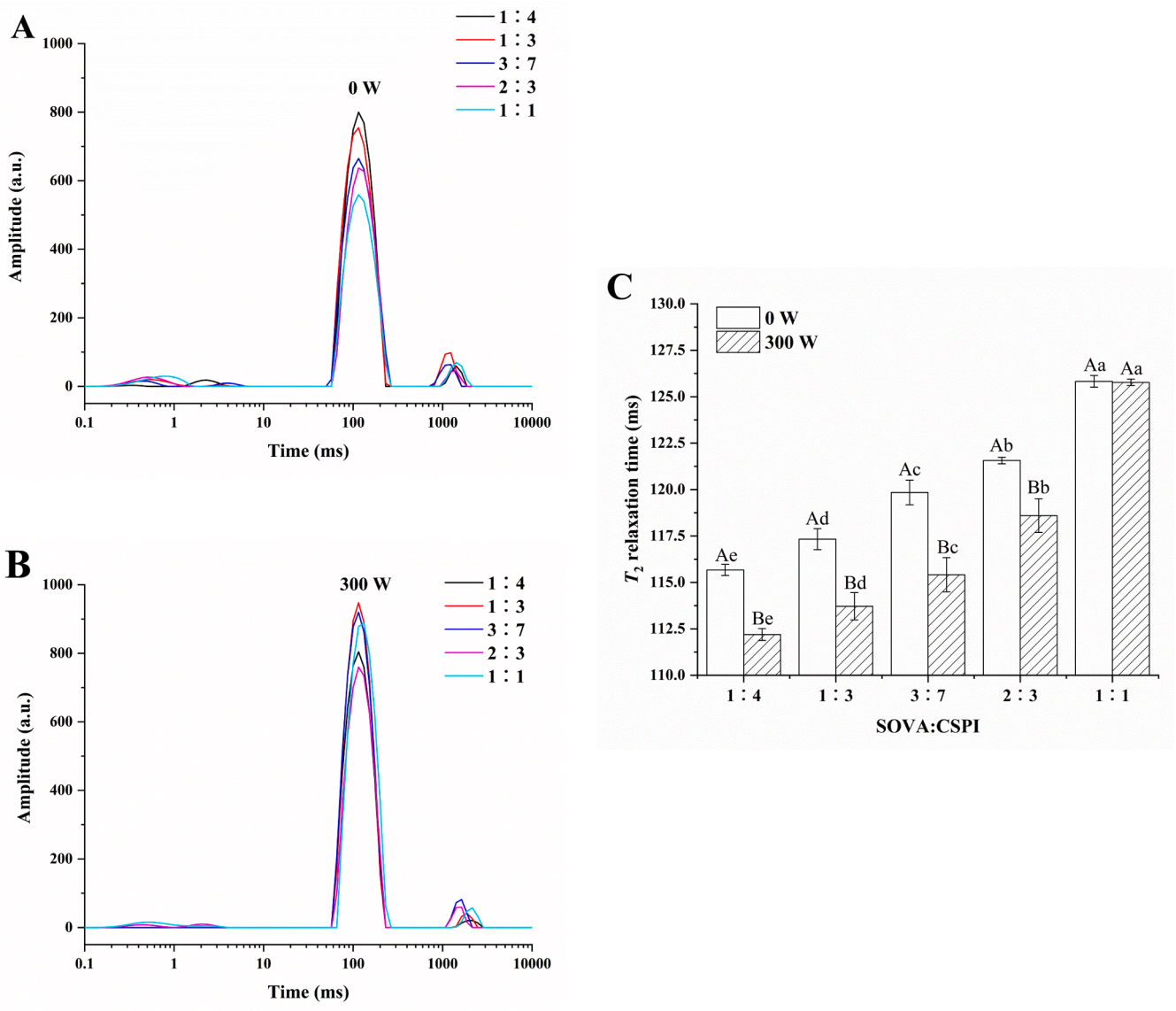


Fig. 3. Changes in the T_2 of different ratios of SOVA-CSPI hybrid gels untreated or treated with ultrasound. (A-B: multi-component treatment; C: single-component treatment). Different uppercase letters (A-B) indicated significant differences between samples untreated or treated with ultrasound ($P < 0.05$), and different lowercase letters (a-e) indicated significant differences between samples of different ratios ($P < 0.05$).

3.5. T_2 analysis

The state of a gel can be indirectly reflected by the LF-NMR technique, as it was commonly used to study the type of water in the gel (PerezMateos, Lourenco, Montero, & Borderias, 1997). In general, there were three states of water molecules present in the system of gel: T_{21} (1–10 ms) was classified as bound water, T_{22} (10–100 ms) represented non-flowable water, T_{23} (100–1000 ms) was considered as free water (Srisai, Pansawat, Rattanaporn, & Limpisophon, 2023). After multi-component processing of the data, the variations of T_2 in the hybrid gels untreated or treated with ultrasound were shown in Fig. 3A-B. As presented, T_{21}, T_{22}, and T_{23} were observed in the hybrid gels untreated or treated with ultrasound, and T_{22} was the dominant one, indicating there were three states of water in the hybrid gels, of which bound water was predominant. As presented in Fig. 3C, with the increase of SOVA addition, the T_2 relaxation time gradually increased ($P < 0.05$). According to what was reported by Li, Zhang, et al. (2018), the water was more tightly bound to proteins when the relaxation time was shorter. Therefore, the binding of water to proteins gradually became weaker with the increase of SOVA addition, which was also proved by the results of WHC (Fig. 1D). As discussed earlier, the electrostatic repulsion formed between the molecules of protein gradually declined with the growth of SOVA addition (Fig. 1A), which promoted the agglomeration of protein molecules. The gap in the network of hybrid gel became smaller with the increase of SOVA addition due to the agglomeration of protein molecules, thus leading to the reduction in the ability of the protein to bind water.

As shown in Fig. 3A-B, the T_{22} peak area increased after being treated with ultrasound, suggesting an increase in the content of non-flowable water. This might be due to that the water molecules in SOVA and CSPI were not entirely involved in the development of the gel, and the ultrasonic treatment facilitated the involvement of water in the formation of the gel. Except for the ratio of 1:1, the T_2 relaxation time tended to decrease ($P < 0.05$) after ultrasonic treatment. It indicated that the distribution of water molecules in the SOVA-CSPI hybrid gel network was improved under ultrasonic treatment, thus the water was promoted to bind with proteins.

3.6. Variations of the surface hydrophobicity in SOVA-CSPI hybrid gels

Not only the conformational changes but also the functional properties of proteins were tightly correlated with the surface hydrophobicity. As depicted in Fig. 4A, the surface hydrophobicity all showed a pronounced decrease ($P < 0.05$) with the growth of SOVA addition, regardless of whether the SOVA-CSPI hybrid gels were treated with ultrasound or not. The surface hydrophobicity decreased by 37.6 % (0 W) and 51.1 % (300 W) for SOVA/CSPI of 1:4 compared to the surface hydrophobicity for SOVA/CSPI of 1:1. The decrease in the surface hydrophobicity might be attributed that the hydrophobic amino acid residues of SOVA-CSPI being covered by proteins, changing the conformation of the protein gel. From the findings in terms of ζ-potential (Fig. 1A), the electrostatic repulsion formed between the molecules of protein in SOVA-CSPI hybrid gels declined with the growth of SOVA addition, and the protein molecules tended to aggregate, so the hydrophobic amino acid residues on the surface of protein might be covered, thus reducing the surface hydrophobicity.

The surface hydrophobicity showed an increased trend after the hybrid gels were treated with ultrasound. Some studies have shown that the strong cavitation effect produced by ultrasonic treatment exposed the hydrophobic amino residues inside the protein, leading to an increase in the surface hydrophobicity (Hu et al., 2013; Xue, Tu, et al., 2021). The hydrophobic amino residues buried inside the protein in SOVA-CSPI might be exposed under the treatment of ultrasound, thus increasing the surface hydrophobicity after the hybrid gels were treated with ultrasound. The ultrasonic treatment effectively enhanced the surface hydrophobicity and promoted the hydrophobic interactions

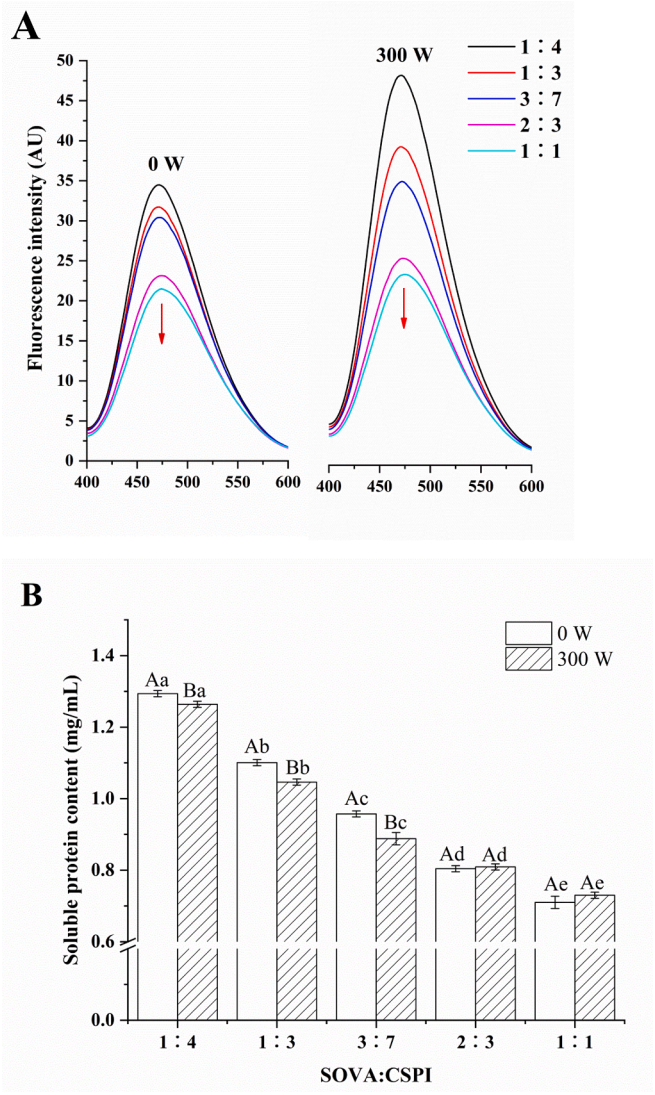


Fig. 4. Changes in the surface hydrophobicity (A) and the soluble protein content (B) of different ratios of SOVA-CSPI hybrid gels untreated or treated with ultrasound. Different uppercase letters (A-B) indicated significant differences between samples untreated or treated with ultrasound ($P < 0.05$), and different lowercase letters (a-e) indicated significant differences between samples of different ratios ($P < 0.05$).

between protein molecules, resulting in an increase in the cross-linking between protein molecules. This might be the reason for the enhancement in the stiffness (Fig. 1B) and cohesiveness (Fig. 1C) of SOVA-CSPI hybrid gels after being treated with ultrasound.

3.7. Variations of the soluble protein content in SOVA-CSPI hybrid gels

The extent of degradation and aggregation of the protein, which was highly correlated with the gelling properties of the protein, can be reflected by the protein solubility (Jun, Mu, Hui, Obadi, Chen, & Bin, 2020). As shown in Fig. 4B, with the growth of SOVA addition, the soluble protein content presented a remarkable downward trend ($P < 0.05$), regardless of whether the SOVA-CSPI hybrid gels were treated with ultrasound or not. Combined with the findings in terms of ζ-potential (Fig. 1A), with the growth of SOVA addition, proteins were promoted to aggregate due to the reduction in the electrostatic repulsion formed between the molecules of protein. And the hydrophilic groups in protein were wrapped due to the aggregation of the protein, thus causing a reduction in the soluble protein content. After being treated

with ultrasound, the soluble protein content of the hybrid gels presented a decreasing trend ($P < 0.05$). This might be due to that more soluble aggregates were embedded in the CSPI network structure under the ultrasonic treatment, thus reducing the solubility of the proteins.

3.8. Variations of the intermolecular forces in SOVA-CSPI hybrid gels

The gelation of protein was a complicated process in which the intermolecular forces showed a change, eventually leading to protein aggregation and gel formation (Totosaus, Montejano, Salazar, & Guerrero, 2002). To further investigate the formation mechanism of SOVA-CSPI hybrid gels and to make sure the intermolecular forces participated in maintaining the structure of SOVA-CSPI hybrid gels, the hybrid gels were dissolved in four different denaturing solvents.

As shown in Fig. 5, most of the SOVA-CSPI hybrid gels with or without ultrasonic treatment were dissolved in the solvents of S1, S3, and S4, comprising more than 95 %, implying that the formation of the hybrid gels was predominantly dominated by ionic bonds, hydrophobic interactions, and disulfide bonds. And the solubility of the hybrid gels in the solvent of S2 was low, implying that the contribution of hydrogen bonds to the formation of the hybrid gel was low. And the intermolecular forces showed a pronounced change ($P < 0.05$) with the growth of SOVA addition. The solubility of the hybrid gels with or without ultrasonic treatment in the solvent of S3 declined with the growth of SOVA addition, suggesting a decrease in the hydrophobic interactions. This result was similar to the result of changes in the surface hydrophobicity (Fig. 4A). As discussed earlier, with the increase of SOVA addition, the protein molecules tended to aggregate due to the decrease in the electrostatic repulsion formed between the molecules of protein (as shown in Fig. 1A), leading to the burial of hydrophobic amino acid residues, thus resulting in the decrease in the hydrophobic interactions. While the solubility of the hybrid gels with or without ultrasonic treatment in the solvent of S4 increased with the growth of SOVA addition, indicating the increase in the disulfide bonds. According to what was reported by (Zhao et al., 2016), there were four free sulfhydryl groups and a disulfide bond buried inside the ovalbumin molecule. With the growth of SOVA addition, the content of OVA in the SOVA-CSPI hybrid gels also increased, so more disulfide bonds might be generated through the oxidation reaction of free sulfhydryl. It has been shown that disulfide bonds might be tightly related to the strength of the gel, and the higher the content of disulfide bonds, the greater the hardness of the gel (Totosaus, Montejano, Salazar, & Guerrero, 2002). This might be one of the reasons for the enhancement in the stiffness of the SOVA-CSPI hybrid gels with the growth of SOVA addition (Fig. 1B).

After the treatment of ultrasound, the solubility of the hybrid gels in the solvent of S3 increased ($P < 0.05$), which might be owing to that the hydrophobic amino residues hidden inside the protein were exposed under the ultrasonic treatment, thus increasing the hydrophobic interactions. However, the solubility of the hybrid gels in the solvent of S4 tended to decrease after the treatment of ultrasound, which might be due to the exposed sulfhydryl groups being buried inside the protein molecules again under the ultrasonic treatment, thus reducing the formation of disulfide bonds.

3.9. The proposed mechanism of the influence of ultrasonic treatment on SOVA-CSPI hybrid gels

During the heating process, OVA preferentially aggregated to form random aggregates. Then those aggregates interacted with CSPI and filled the porous network structure of CSPI to form a hybrid gel. Combined with the findings in the experiments, Fig. 6 presented the hypothetical mechanism diagram of the influence of ultrasonic treatment on SOVA-CSPI hybrid gels. The cavitation effect generated by ultrasound exposed the hidden hydrophobic amino residues inside the proteins, increasing the hydrophobic interactions (Hu et al., 2013; Xue, Tu, et al., 2021). After being treated with ultrasound, more water molecules

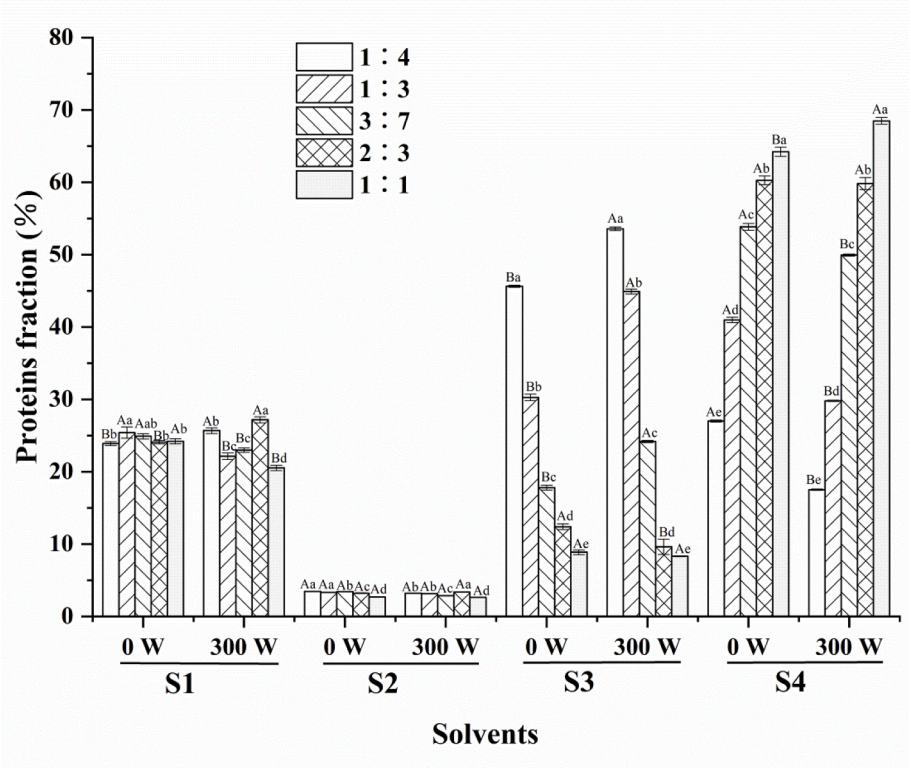


Fig. 5. Changes in the intermolecular forces of different ratios of SOVA-CSPI hybrid gels untreated or treated with ultrasound. Different uppercase letters (A-B) indicated significant differences between samples untreated or treated with ultrasound ($P < 0.05$), and different lowercase letters (a-e) indicated significant differences between samples of different ratios ($P < 0.05$).

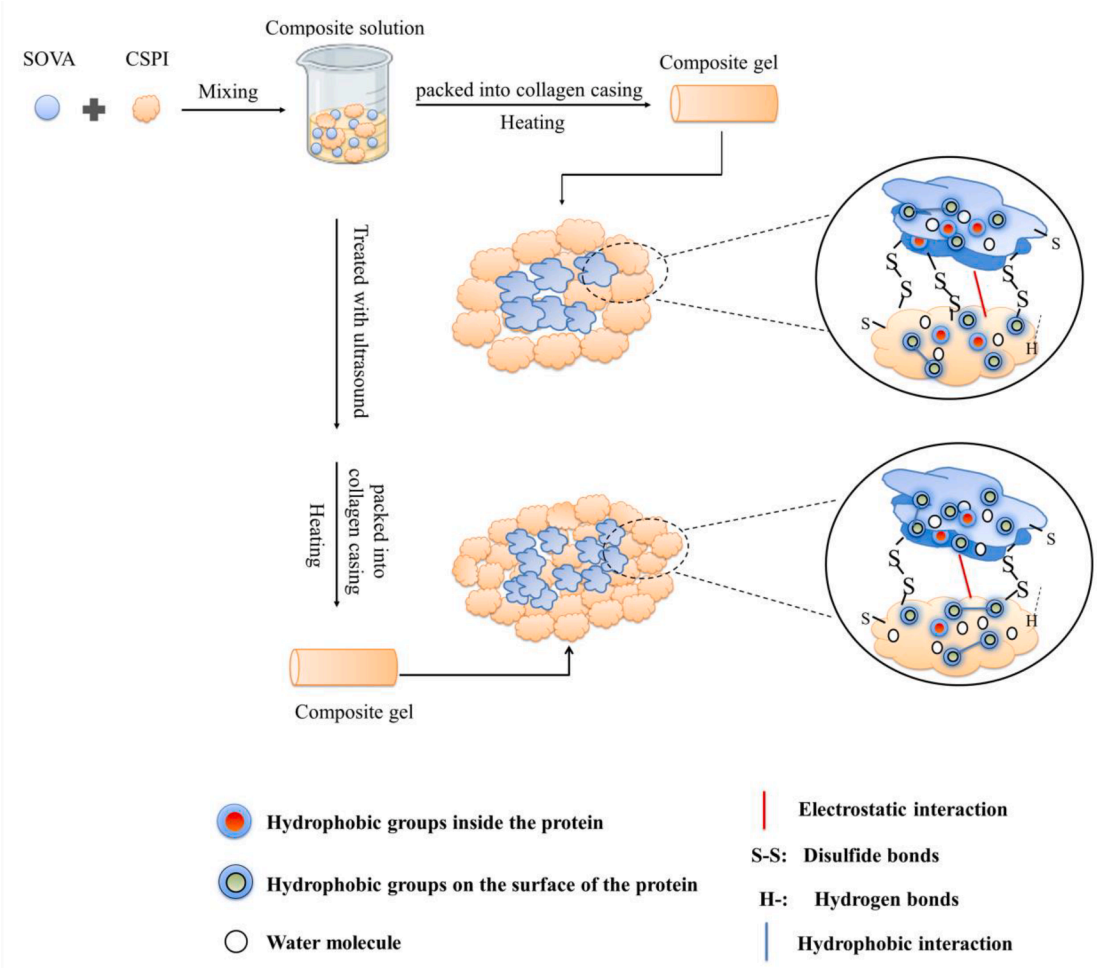


Fig. 6. The proposed mechanism of the influence of ultrasonic treatment on SOVA-CSPI hybrid gels.

participated in the gel formation, and the binding ability of water molecules to proteins was strengthened. Under the treatment of ultrasound, the exposed sulfhydryl groups were buried inside the protein molecules again, leading to the decline in the content of disulfide bonds. In general, the properties and structures of SOVA-CSPI hybrid gels were enhanced under ultrasonic treatment.

4. Conclusion

It was found that the addition of SOVA to CSPI solution can form a stable hybrid gel after heating treatment. With the growth of SOVA addition, the ζ-potential in the absolute value of the SOVA-CSPI hybrid gels declined, suggesting that the electrostatic repulsion formed between the molecules of protein reduced and the proteins tended to aggregate. The unfolding of proteins was hindered and the binding of protein-water decreased due to the reduced electrostatic repulsion, leading to a decrease in the cohesiveness and WHC. In addition, the surface hydrophobicity and soluble protein content declined with the growth of SOVA addition, further indicating the aggregation of different proteins. The intermolecular forces maintaining the SOVA-CSPI hybrid gels were mainly hydrophobic interactions and disulfide bonds. After the treatment of ultrasound, the properties of SOVA-CSPI hybrid gels were improved. The interaction between protein and water molecules was enhanced by the ultrasonic treatment, thus improving the WHC of SOVA-CSPI hybrid gels. Additionally, under the treatment of ultrasound, more functional groups were exposed, resulting in increased cross-linking and aggregation of proteins, thus leading to the increase in T_{22} and the enhancement of hydrophobic interactions. The hybrid gels exhibited a denser network structure after ultrasonic treatment, thus increasing the stability and resistance to extrusion of the hybrid gels.

CRediT authorship contribution statement

Ji'en Tan: Writing – original draft, Formal analysis, Data curation. **Wei Qiu:** Data curation, Conceptualization. **Na Wu:** Writing – review & editing. **Lilan Xu:** Writing – review & editing. **Shuping Chen:** Writing – review & editing. **Yao Yao:** Writing – review & editing. **Mingsheng Xu:** Writing – review & editing. **Yan Zhao:** Writing – review & editing, Validation, Resources, Project administration. **Yonggang Tu:** Validation, Supervision, Resources, 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.

Acknowledgments

We gratefully acknowledge the financial support provided by the National Natural Science Foundation of China (Grant No. 32102011), Jiangxi Provincial Key Research and Development Project

(20232BBF60025), Training Project of High-level and High-skilled Leading Talents of Jiangxi Province (29202300002) and the Natural Science Foundation of Jiangxi Province (20212BAB215019).

References

Chang, C., Niu, F., Gu, L., Li, X., Yang, H., Zhou, B., Wang, J., Su, Y., & Yang, Y. (2016). Formation of fibrous or granular egg white protein microparticles and properties of the integrated emulsions. *Food Hydrocolloids, 61*, 477–486. https://doi.org/10.1016/j.foodhyd.2016.06.002

Ding, Z., Li, S., & Cao, X. (2014). Separation of lysozyme from salted duck egg white by affinity precipitation using pH-responsive polymer with an L-thyroxin ligand. *Separation and Purification Technology, 138*, 153–160. https://doi.org/10.1016/j.seppur.2014.10.021

Du, M., Sun, Z., Liu, Z., Yang, Y., Liu, Z., Wang, Y., Jiang, B., Feng, Z., & Liu, C. (2022). High efficiency desalination of wasted salted duck egg white and processing into food-grade pickering emulsion stabilizer. *LWT-Food Science and Technology, 161*, Article 113337. https://doi.org/10.1016/j.lwt.2022.113337

Han, T., Xue, H., Hu, X., Li, R., Liu, H., Tu, Y., & Zhao, Y. (2022). Combined effects of NaOH, NaCl, and heat on the gel characteristics of duck egg white. *LWT-Food Science and Technology, 159*. https://doi.org/10.1016/j.lwt.2022.113178

Higuera-Barraza, O. A., Del Toro-Sanchez, C. L., Ruiz-Cruz, S., & Marquez-Rios, E. (2016). Effects of high-energy ultrasound on the functional properties of proteins. *Ultrasonics Sonochemistry, 31*, 558–562. https://doi.org/10.1016/j.ultsonch.2016.02.007

Hu, H., Cheung, I. W., Pan, S., & Li-Chan, E. C. (2015). Effect of high intensity ultrasound on physicochemical and functional properties of aggregated soybean β-conglycinin and glycinin. *Food Hydrocolloids, 45*, 102–110. https://doi.org/10.1016/j.foodhyd.2014.11.004

Hu, H., Fan, X., Zhou, Z., Xu, X., Fan, G., Wang, L., Huang, X., Pan, S., & Zhu, L. (2013). Acid-induced gelation behavior of soybean protein isolate with high intensity ultrasonic pre-treatments. *Ultrasonics Sonochemistry, 20*(1), 187–195. https://doi.org/10.1016/j.ultsonch.2012.07.011

Jun, S., Mu, Y., Hui, J., Obadi, M., Chen, Z., & Bin, X. (2020). Effects of single- and dual-frequency ultrasound on the functionality of egg white protein. *Journal of Food Engineering, 277*. https://doi.org/10.1016/j.jfoodeng.2020.109902

Lan, Q., Li, L., Dong, H., Wu, D., Chen, H., Lin, D., Qin, W., Yang, W., Vasanthan, T., & Zhang, Q. (2019). Effect of soybean soluble polysaccharide on the formation of glucono-δ-lactone-induced soybean protein isolate gel. *Polymers (Basel), 11*(12). https://doi.org/10.3390/polym11121997

Li, J., Li, X., Wang, C., Zhang, M., Xu, Y., Zhou, B., Su, Y., & Yang, Y. (2018). Characteristics of gelling and water holding properties of hen egg white/yolk gel with NaCl addition. *Food Hydrocolloids, 77*, 887–893. https://doi.org/10.1016/j.foodhyd.2017.11.034

Li, J., Zhang, M., Chang, C., Gu, L., Peng, N., Su, Y., & Yang, Y. (2018). Molecular forces and gelling properties of heat-set whole chicken egg protein gel as affected by NaCl or pH. *Food Chemistry, 261*, 36–41. https://doi.org/10.1016/j.foodchem.2018.03.079

Li, R., Xue, H., Gao, B., Liu, H., Han, T., Hu, X., Tu, Y., & Zhao, Y. (2022). Physicochemical properties and digestibility of thermally induced ovalbumin–oil emulsion gels: Effect of interfacial film type and oil droplets size. *Food Hydrocolloids, 131*, Article 107747. https://doi.org/10.1016/j.foodhyd.2022.107747

Lin, H.-F., Lu, C.-P., Hsieh, J.-F., & Kuo, M.-I. (2016). Effect of ultrasonic treatment on the rheological property and microstructure of tofu made from different soybean cultivars. *Innovative Food Science & Emerging Technologies, 37*, 98–105. https://doi.org/10.1016/j.ifset.2016.08.013

Lu, Y., Zhu, Y., Ye, T., Nie, Y., Jiang, S., Lin, L., & Lu, J. (2022). Physicochemical properties and microstructure of composite surimi gels: The effects of ultrasonic treatment and olive oil concentration. *Ultrasonics Sonochemistry, 88*. https://doi.org/10.1016/j.ultsonch.2022.106065

Niu, F., Kou, M., Fan, J., Pan, W., Feng, Z. J., Su, Y., Yang, Y., & Zhou, W. (2018). Structural characteristics and rheological properties of ovalbumin-gum arabic complex coacervates. *Food Chemistry, 260*, 1–6. https://doi.org/10.1016/j.foodchem.2018.03.141

PerezMateos, M., Lourenco, H., Montero, P., & Borderias, A. J. (1997). Rheological and biochemical characteristics of high-pressure- and heat-induced gels from blue whiting (Micromesistius poutassou) muscle proteins. *Journal of Agricultural and Food Chemistry, 45*(1), 44–49. https://doi.org/10.1021/jf960185m

Renkema, J. M. S., & van Vliet, T. (2002). Heat-induced gel formation by soy proteins at neutral pH. *Journal of Agricultural and Food Chemistry, 50*(6), 1569–1573. https://doi.org/10.1021/jf010763l

Shao, J.-H., Deng, Y.-M., Song, L., Batur, A., Jia, N., & Liu, D.-Y. (2016). Investigation the effects of protein hydration states on the mobility water and fat in meat batters by LF-NMR technique. *LWT-Food Science and Technology, 66*, 1–6. https://doi.org/10.1016/j.lwt.2015.10.008

Sow, L. C., Chong, J. M. N., Liao, Q. X., & Yang, H. (2018). Effects of κ-carrageenan on the structure and rheological properties of fish gelatin. *Journal of Food Engineering, 239*, 92–103. https://doi.org/10.1016/j.jfoodeng.2018.05.035

Sow, L. C., Toh, N. Z. Y., Wong, C. W., & Yang, H. (2019). Combination of sodium alginate with tilapia fish gelatin for improved texture properties and nanostructure modification. *Food Hydrocolloids, 94*, 459–467. https://doi.org/10.1016/j.foodhyd.2019.03.041

Srisai, P., Pansawat, N., Rattanaporn, K., & Limpisophon, K. (2023). Textural and physicochemical properties of threadfin bream surimi gels prepared with salted duck egg white as substitute for hen egg white. *International Journal of Food Science and Technology, 58*(3), 1098–1107. https://doi.org/10.1111/ijfs.16251

Taha, A., Hu, T., Zhang, Z., Bakry, A. M., Khalifa, I., Pan, S., & Hu, H. (2018). Effect of different oils and ultrasound emulsification conditions on the physicochemical properties of emulsions stabilized by soy protein isolate. *Ultrasonics Sonochemistry, 49*, 283–293. https://doi.org/10.1016/j.ultsonch.2018.08.020

Tan, E. S., Ying-Yuan, N., & Gan, C. Y. (2014). A comparative study of physicochemical characteristics and functionalities of pinto bean protein isolate (PBPI) against the soybean protein isolate (SPI) after the extraction optimisation. *Food Chemistry, 152*, 447–455. https://doi.org/10.1016/j.foodchem.2013.12.008

Tan, J. E., Deng, C., Yao, Y., Wu, N., Du, H., Xu, M., Chen, S., Zhao, Y., & Tu, Y. (2022). Effects of different copper salts on the physicochemical properties, microstructure and intermolecular interactions of preserved egg white. *Food Chemistry*. , Article 134756. https://doi.org/10.1016/j.foodchem.2022.134756

Totosaus, A., Montejano, J. G., Salazar, J. A., & Guerrero, I. (2002). A review of physical and chemical protein-gel induction. *International Journal of Food Science and Technology, 37*(6), 589–601. https://doi.org/10.1046/j.1365-2621.2002.00623.x

Wagner, J. R., Sorgentini, D. A., & Anon, M. C. (2000). Relation between solubility and surface hydrophobicity as an indicator of modifications during preparation processes of commercial and laboratory-prepared soy protein isolates. *Journal of Agricultural and Food Chemistry, 48*(8), 3159–3165. https://doi.org/10.1021/jf990823b

Wen, P., Xia, C., Zhang, L., Chen, Y., Xu, H., Cui, G., & Wang, J. (2023). Effects of different dry heating temperatures on the spatial structure and amino acid residue side-chain oxidative modification of soybean isolated proteins. *Food Chemistry, 405*. https://doi.org/10.1016/j.foodchem.2022.134795

Wu, C., Wang, J., Yan, X., Ma, W., Wu, D., & Du, M. (2020). Effect of partial replacement of water-soluble cod proteins by soy proteins on the heat-induced aggregation and gelation properties of mixed protein systems. *Food Hydrocolloids, 100*. https://doi.org/10.1016/j.foodhyd.2019.105417

Xin, Y., Chai, M., Chen, F., Hou, Y., Lai, S., & Yang, H. (2021). Comparative study on the gel properties and nanostructures of gelatins from chicken, porcine, and tilapia skin. *Journal of Food Science, 86*(5), 1936–1945. https://doi.org/10.1111/1750-3841.15700

Xu, L., Zhao, Y., Xu, M., Yao, Y., Nie, X., Du, H., & Tu, Y.-G. (2017). Effects of salting treatment on the physicochemical properties, textural properties, and microstructures of duck eggs. *PLoS One, 12*(8), Article e0182912. https://doi.org/10.1371/journal.pone.0182912

Xu, L., Zhao, Y., Xu, M., Yao, Y., Nie, X., Du, H., & Tu, Y. (2018). Changes in aggregation behavior of raw and cooked salted egg yolks during pickling. *Food Hydrocolloids, 80*, 68–77. https://doi.org/10.1016/j.foodhyd.2018.01.026

Xue, H., Liub, H., Wu, N., Zhang, G., Tua, Y., & Zhao, Y. (2022). Improving the gel properties of duck egg white by synergetic phosphorylation/ultrasound: Gel properties, crystalline structures, and protein structure. *Ultrasonics Sonochemistry, 89*. https://doi.org/10.1016/j.ultsonch.2022.106149

Xue, H., Tu, Y., Zhang, G., Xin, X., Hu, H., Qiu, W., Ruan, D., & Zhao, Y. (2021). Mechanism of ultrasound and tea polyphenol assisted ultrasound modification of egg white protein gel. *Ultrasonics Sonochemistry, 81*. https://doi.org/10.1016/j.ultsonch.2021.105857

Xue, H., Xu, M., Liao, M., Luo, W., Zhang, G., Tu, Y., & Zhao, Y. (2021). Effects of tea and illicium verum braise on physicochemical characteristics, microstructure, and molecular structure of heat-induced egg white protein gel. *Food Hydrocolloids, 110*. https://doi.org/10.1016/j.foodhyd.2020.106181

Yang, D., Gao, S., & Yang, H. (2020). Effects of sucrose addition on the rheology and structure of iota-carrageenan. *Food Hydrocolloids, 99*, Article 105317. https://doi.org/10.1016/j.foodhyd.2019.105317

Yu, L., Xiong, C., Li, J., Luo, W., Xue, H., Li, R., Tu, Y., & Zhao, Y. (2020). Ethanol induced the gelation behavior of duck egg whites. *Food Hydrocolloids, 105*. https://doi.org/10.1016/j.foodhyd.2020.105765

Zhang, M., Li, J., Su, Y., Chang, C., Li, X., Yang, Y., & Gu, L. (2019). Preparation and characterization of hen egg proteins-soybean protein isolate composite gels. *Food Hydrocolloids, 97*. https://doi.org/10.1016/j.foodhyd.2019.105191

Zhao, Y., Chen, Z., Li, J., Xu, M., Shao, Y., & Tu, Y. (2016). Formation mechanism of ovalbumin gel induced by alkali. *Food Hydrocolloids, 61*, 390–398. https://doi.org/10.1016/j.foodhyd.2016.04.041